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The 9th International Conference on Agriculture and Environmental Sciences

2023

Preface

Welcome to the Proceedings of the 9th International Conference on Agriculture and Environmental Sciences (CAES-2023) 6-7 March 2023, Tikrit, Iraq.

The CAES-2023 addresses conference is established to provide an ideal platform for researchers to share views and experiences in Environmental areas and discussion on food, agriculture, and how technology is effectively employed for sustainable development of food and agriculture. It is aimed to bring researchers, academicians, scientists, students, and practitioners together to participate and present the latest research findings, developments, and applications related to various aspects of agriculture engineering, organic agriculture, agribusiness, animal nutrition, animal production, veterinary Science, food science and technology, food safety, food security and sovereignty, IT for Agriculture, renewable energy and other researches.

We would like to express our sincere gratitude to the Chairman, the distinguished keynote speakers, as well as all the participants. We also want to thank the publisher for publishing the proceedings. May the readers could enjoy the gain some valuable knowledge from it. We are expecting more and more experts and scholars from all over the world to join this international event next year.

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Best regards,

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The way toAchieve sustainable Agriculture andFace Climate Change

Quantifying Available soil water by Least Limiting Water Range and Integral Water Capacity Approaches for a Gypsiferous Soil

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Abstract. Laboratory experiments to decide the available soil water quantitatively (PAW) consistent with the principles of least water resistance for plant increase (LLWR) and integrated water capacity (IWC) fashions of soils with different gypsum content material (61 - 443 g kg^{-1}). Different soil samples with a gypsum percent of [105 (G 2), 154 (G 3), 207 (G 4), 253 (G 5) and 355 (G 6) g kg^{-1}] have been prepared by using mixing with a gypsum content 61 (G1) g kg^{-1} for document 0-10 and subsurface soil with gypsum content (G 7) 443 g kg^{-1} for subculture 60-100 hundred quantity gypsum soil, use SAW Cal and RETC software to obtain values of prepared soil water PAW_{10} and PAW $_{33}$ Water Determinant $LLWR_{10}$ and $LLWR_{33}$ at Tensile Strengths of 10 and 33 kPa, Integral Water Capacity IWC, Soil Molecule, Integral Viability of Ready Soil Water EI $_{(PAW10)}$ and E I $_{(PAW33)}$ Integral for a Water Defined Range, E I $_{(LLWR10)}$ and E I $_{(LLWR33)}$ Integral ability of integration E I $_{(IWC)}$, and the following results have been evaluated : The values of water available to the plant increased with the increase in the soil content of gypsum at a tensile strength of 10 kPa, compared to a tensile strength of 33 kPa, as it reached 0.025 $cm^3cm^{-3,}$ Gypsum content material 61 g $kg^{-1}$and this difference increased with the increase in the percentage of gypsum to 440 g kg, when it reached 0.046 cm^3cm^{-3} as well as for ($LLWR_{10}$) and ($LLWR_{33}$) and IWC, as for the integrated energy of water As for the incorporated strength of the plant EI (PAW) As the values of the included electricity of water available to the plant at EI (PAW_{10}) have been less than EI (PAW_{33}) in keeping with the difference within the soil content of gypsum .It is referred to that the distinction between the values of EI (PAW_{10}) and the values of EI (PAW_{33}) is predicted from the receiver E I $_{(LLWR10)}$ EI $_{(LLWR33)}$ EI $_{(ICW)}$.

Keywords. Plant Available Water, Least Limit Water Rangr, Integral Water Capasity, Integral energy.

1. Introduction

The significance of estimating available water for flora (PAW) comes because of its direct effect on the surroundings, soil, and plant increase alike, and because of the significance of water for flora, several new concepts have been proposed to deduce it to begin estimating it, and among these concepts is the least particular range of water (LLWR) and Integrated Water Capacity (IWC) Concepts of LLWR and IWC Soil indicators in bodily soil It is easy to use for carried out purposes, measuring

soil mechanical houses and field or laboratory to reach information of soil in bodily soil [1]. The idea of PAW is the amount of water held among the sector ability (θ_{fc}) and the permanent wilting factor ($\theta_{\_pwp}$) and at values 330 and 100 kPa, which is the most established.
[2], offered the concept of incorporated water potential (IWC) to determine the available soil water that works within the bodily homes of the soil and the water absorption with the aid of the plant [1]. [3], determined negative correlations between LLWR, IWC, bulk density (BD), and reference bulk density (BD_{ref}), It indicates a positive relationship Between LLWR and IWC on soil physical quality LLWR values indicate successful soil management, while low or limited LLWR values indicate poor soil management [4,5]. [6], proposed incorporated energy (EI) a concept to estimate the strength needed by a plant to take in a specific amount of soil water. LLWR values decrease whilst soil content material of clay is extended whilst soil compaction is high [7], among [8], confirmed that plant increase is extra environmental in soils with restrained LLWR as compared with excessive LLWR.

2. Materials and Methods

2.1. Gypsum Soil Sampling Site

Soil samples have been taken from a gypsum soil bed at the research station of the College of Agriculture / University of Tikrit positioned at longitude 43^{O} 38' 23"E and latitude $34^{O}40'\ 48''$hight'N 250 m above sea stage. Cheese turned into extracted from the floor soil of the soil of plots 0- 10 cm, the proportion of which was 61 g / kg^{-1} (G 1) and the subsurface gypsum horizon for a depth of 60 - a hundred cm, the share of gypsum turned into 443 g / kg^{-1} (G 7). Soil samples were air dried, then ground and exceeded through a sieve with a diameter of 2 mm. Then, distinctive samples were prepared with a ratio of gypsum a hundred and five[(G2) 154 (G3), 207 (G4), 253 (G5), and 355 (G6) gkg^{-1}]. By blending floor soil samples (G 1) and sub-floor soil (G 7) prepared soil samples (G 7 - G 1) by means of spraying to the boundaries of two-thirds of the field capacity, then incubated in a sealed field with non-stop stirring for two months, and after completion Soil samples had been aerobic, then floor and surpassed via a sieve with a diameter of 2 mm, and then saved in pre-tested boxes.

2.2. Calculation SAW, LLWR and IWC

For the calculation of LLWR, IWC and their corresponding HI values, the plant-geared up water (PAW) is calculated from the distinction among the moisture content of the sphere ability (FC) at 330 and one hundred hPa tension and the everlasting wilting point (PAW) at 15,000 hPa tension, floor at the top restriction of capacity. In area stresses of 330 and one hundred hPa, the PAW was calculated on the premise of PAW 100 and PAW 330, respectively [1,8].
The upper restrict of the LLWR is the sphere potential moisture content material (FC) at tensile electricity of 100 and 330 hPa or at 10% porosity (air porosity), the lower restriction of the LLWR is the permanent wilting factor moisture content (PWP) at tensile energy of 15,000 hPa or the soil penetration resistance at Tensile 2 MPa, installation at the most field capacitance (FC) LLWR become calculated with the aid of LLER a hundred and LLWR 330 respectively [1,8].

$$LLWR = UL - LL \tag{1}$$

Since: - UL = upper restriction of LLWR . LL = lower restriction of LLWR
The essential water potential IWC turned into calculated from the subsequent equation (2).

$$IWC = \int_0^{\infty} (\prod_{i=1}^{m} \omega i(h)) C(h) dh \tag{2}$$

Retrieving 15,000 specific data to get greater records 0-15000hPaIncremental (h) tensile energy of 1 hPa, calculating the integration in the above equation by means of the trapezoidal approach by way of dividing the whole moisture tensile range right into a massive quantity of increments [9]. dh and ωi(h) represent a weighted characteristic of soil physical homes from (1 to m) as a feature of h .$\prod as$ The represents all values of The weighted function wi(h) that multiplies in the IWC domain and represents C(h) the differential German capacitance. Ei (h) changed into calculated from the subsequent equation:

$$E\ i\ (h) = \omega\ i\ (h)\ C\ (h) \tag{3}$$

As the values of ωi(h) same to when there are integral determinants of water uptake by the plant and it increases continuously to 1 when there are no determinants of water uptake by the plant. The speedy percolation of soil water due to gravity and poor soil aeration are the overall factors at the moist volume of the soil. As for after the watery soil, the low conductivity of the soil on the high resistivity of the soil are the elements. The values of the burden functions for the four factors were calculated according to the technique utilized by [1,2].

The IWC calculation become unique on the moisture tensile of 1500 kPa, via the quantity of water extracted from the soil at very high tensile strengths, and that is proper through assets to attain an ink source. In addition to the reality that the water conductivity of the local soil is low at high tension and may be unnoticed [10,11].

The values of E_l (J kg^{-1}) for the to be had soil water values may be illustrated by using the PAW, LLWR and IWC values shown to be calculated the use of the evolved equation furnished with the aid of [6,12]:

$$E_l = \frac{1}{10SAW} \int_{hi}^{hf} h \left(\prod_{i=1}^{m} \omega i(h)\right) C(h) dh \quad (4)$$

Since:

h_i = moist (beginning) boundary structural anxiety (hPa).

h_f = dry (final) structural tensile (hPa).

SAW = amount of available soil water (cm^3 cm^{-3}).

10 it unit conversion steady E_l from hpa to joules kg^{-1} .

Structural tensile values at h_i and h_f were used to locate plant water availability (PAW), which may be indicated with FC and PWP values, respectively. The structural tensile values h_i and h_f represent the upper and lower LLWR degrees, respectively.

The values of the weighted characteristic ωi(h) are 1 within the PAW and LLWR ranges and 0 outdoor those stages. The structural stress values at hello and hf required for the IWC calculation can be located when one of the values of ωi(h) at the wet or dry cease becomes 0, respectively [12]. The hydropower EI values for PAW_{10}, PAW $_{33}$, $LLER_{10}$, LLWR $_{33}$ and IWC are represented by means of the values of E I $_{(PAW10)}$, E I $_{(PAW33)}$, E I $_{(LLWR10)}$, E I $_{(LLWR33)}$ and E I $_{(IWC)}$.

3. Results and Discussion

3.1. Effect of Soil Content of Gypsum on Soil Moisture Content and Average Pore Diameters (r)

The pores in the kidney are described via their length, and they represent a part of the plain extent, which is not the plastic materials that comprise the soil material from the soil [13]. Figure 1 suggests examples of the manifestations of the typical version of the studied gypsum, as it's miles visible that the values of the rates of a pore diameter (r) had been received for an boom in growth the capability of the soil to maintain water, as for the soil models G5, G6 and G7 It turned into characterized by means of the predominance of the diameters of the massive pores, which amounted to 0.149, 0.151, and 0 .151μm while saturated, for the role of the massive pores, the transport of water and the aeration of the soil in the continuation of the small streams stored and the dissolved materials in it and their words in the motion and distribution of water and vitamins [14].

When the soil content material of gypsum (G1) become 60 g kg^{-1}, the common effective pore diameters have been 15.08 µm and 4.54µm, the numeric moisture content become 0.371 and 0.333 cm^3 cm^{-3} for tensile strengths of 10 and 33 kPa, respectively, gypsum content (G7) 440 g kg^{-1} average effective diameters were 15.02 and 4.517 µm with volumetric moisture content of 0.256 and 0.204 cm^3 cm^{-3} for tensile strengths of 10 and 33 kPa, respectively. The water content values accelerated at 10 anxiety with an growth in the average effective pore diameters, in assessment to the moisture content material at a 33 tension with a decrease inside the common pore diameters, possibly due to This led to the established order of the distribution in the starting, according to the soil differences, the soil of gypsum, which goes at the lowest, the color of the soil for tensile electricity 33 m in comparison to ten for all studied educational fashions.

It is clear that by increasing the effective pore diameter (greater than 15μm), the moisture content increases with a decrease in the gypsum content of the soil samples . The volume of the soil pores, which change with the change in the soil content of gypsum, which caused the decrease in the moisture content of the soil models - G7. The slope of the curve of the effective pore diameter increased with the increase in the soil content of gypsum. The dominance of effective small pores, as it is noted that the water content decreases at the high water tension values, and the water content increases at the low water tension values with the increase in the gypsum content in the soil Large to medium pores and fine small pores increase with a decrease in the gypsum content in the soil [15]. The presence of effective micro pores (0.2μm) was observed in soils with a low gypsum content and decreased significantly with an increase in the gypsum content in the soil (440g/ kg^{-1}), which in turn leads to a decrease in moisture content, bulk density, physical quality, and nutrient retention in the soil [16].

Table 1. Distribution of effective pore sizes for the studied soil samples.

kPa				μm			
Moisture tensile	**r1i**	**r2i**	**r3i**	**r4i**	**r5i**	**r6i**	**r7i**
0.1	1100	980	1430	1410	1490	1510	1510
1	160	140	140	150	150	150	150
3	50.57	50.2	50.47	50.02	49.54	49.91	49.7
5	29.71	29.32	30.55	29.96	29.51	29.89	29.92
7	21.63	21.44	22.17	21.52	21.29	21.47	21.21
10	15.08	14.83	15.71	15.04	14.78	14.98	15.02
33	4.54	4.458	4.948	4.488	4.497	4.527	4.517
100	1.424	1.456	1.702	1.469	1.503	1.518	1.483
200	0.7612	0.7382	0.833	0.742	0.739	0.7638	0.748
500	0.2927	0.2994	0.355	0.2969	0.301	0.2998	0.308
700	0.2158	0.2039	0.245	0.2125	0.216	0.2083	0.214
1000	0.1478	0.1445	0.176	0.147	0.151	0.1484	0.154
1500	0.0985	0.0998	0.122	0.0994	0.101	0.1015	0.0972

3.2. Effect of Soil Content Material of Gypsum on PAW Values

The concept of plant ready water (PAW) is defined as the amount of water held between discipline capacity ($\theta_{\_fc}$) and everlasting wilting factor ($\theta_{\_pwp}$) and inside the range of moisture tensile values 33 and 1500 kPa, which is the maximum not unusual. Figure 2 shows the effect of the soil content of gypsum on the tensile power values depending on the field capacity at 10 kPa (PAW_{10}) and 33 kPa (PAW_{33}), as extra water geared up to be triumphant within the soil contents ranged from 60 to 350 g kg^{-1}, amounting to 0.084 and 0.134 cm^{-3} cm^{-3} for (PAW_{10}). It was 0.059 and 0.084 $cm^{-3}cm^{-3}$ for(PAW_{33}), respectively, then the water available to plant decreased by increasing the percentage of gypsum in the soil for 350 g kg^{-1}, as it reached 0.114 $cm^{-3}cm^{-3}$ for PAW_{10} and 10.068 $cm^{-3}cm^{-3}$ for PAW_{33} in the soil with a gypsum content of 440 g kg^{-1}.The reason for this can be attributed to the fact that increasing the soil content of gypsum to the limits of 250-350 gm kg^{-1} leads to improving the physical properties of the soil such as bulk density and its reflection in the total porosity of the soil, which increases the ability of the soil to hold water, which increases the water values [17].

The results also show that the values of water available to the plant increased by a range between 0.025 and 0.029 $cm^{-3}cm^{-3}$ for PAW_{10}, compared with PAW_{33} for gypsum soil samples with a gypsum content of 60 and 150 g/kg^{-1}, and this difference increased with an increase in the percentage of gypsum from 200 to 440. g kg^{-1} and as shown in Figure 2, as it reached 0.036 and 0.046 cm^{-3} cm^{-3}, and this can be attributed to the predominance of large soil pore sizes at the expense of small soil pores by increasing the proportion of gypsum from G4 toG, and this coincides with Figure (1) that the increase The percentage of gypsum in the soil leads to a decrease in the availability of water in it due to the lack of organic matter and clay colloids [18,19].

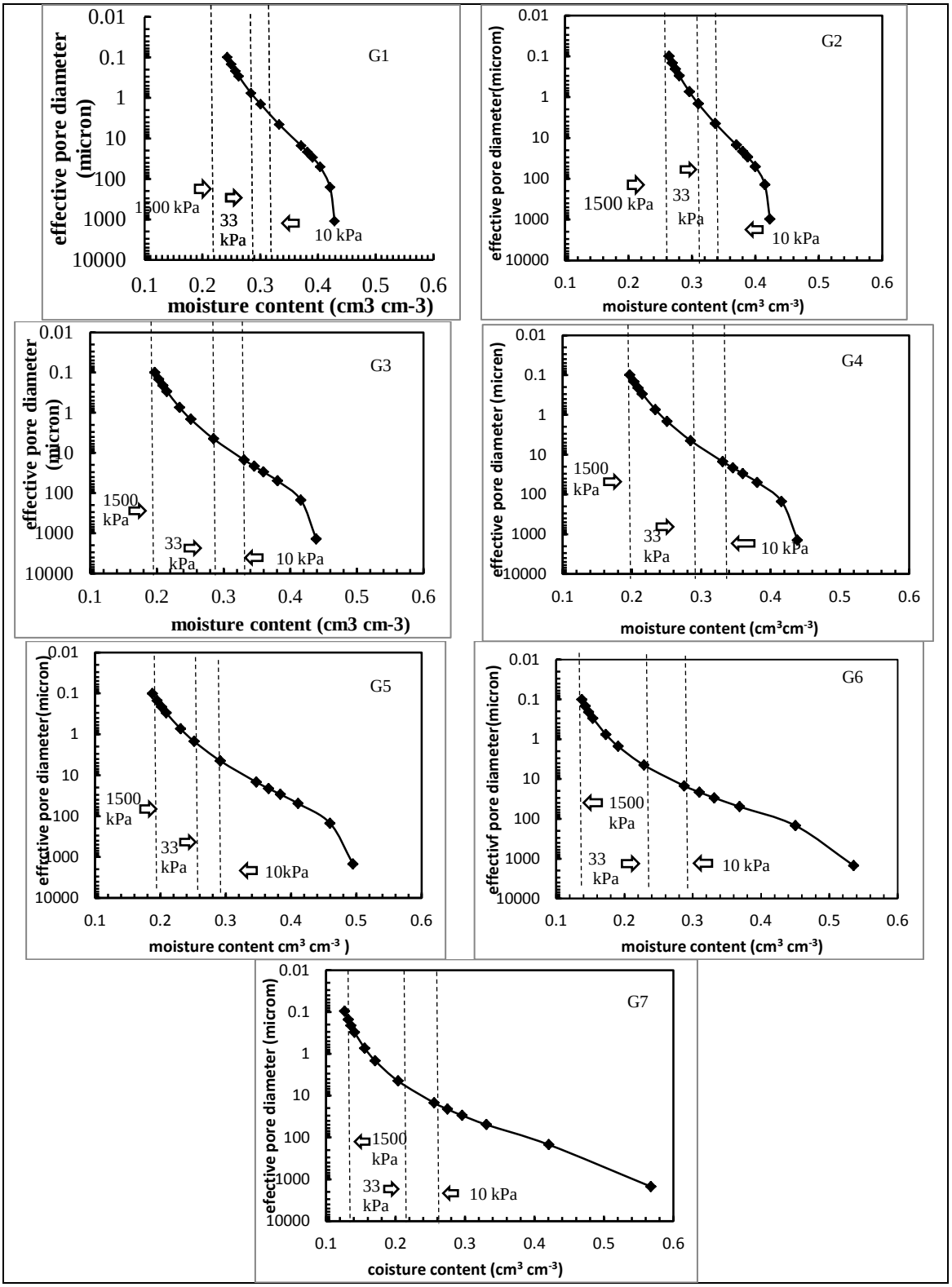

Figure 1. Effect of soil content of gypsum on effective pore size distribution.

3.3. Effect of Soil Gypsum Content Material on the Bottom Water Resistance Degree (LLWR)

The term Minimum Specific Water Range (LLWR) is defined as the range of soil moisture content that is specific for plant growth and associated with the amount of structural stress, moisture and mechanical resistance of the soil and is considered an important indicator of the physical quality of the

soil that is important for plant growth. Figure 3. shows the effect of the soil content of gypsum on the LLWR values at tensile strengths of 10 kPa ($LLWR_{10}$) and 33 kPa,($LLWR_{33}$) as it is noted that these values increased with the increase in the soil content of gypsum from 60 to 350 g kg^{-1}, as it reached 0.014 $cm^{-3}cm^{-3}$ for both $LLWR_{10}$ and $LLWR_{33}$ Soils with a gypsum content of 100 g/kg^{-1}, while the LLWR value increased with the increase of the gypsum content in the soil, as it reached its highest level at the gypsum content of 350 g/kg^{-1}, reaching 0.134 cm^{-3} at $LLWR_{10}$ and 1.084 at $LLWR_{33}$ at the soil content. of gypsum 250 g/kg^{-1}. This is due to the fact that increasing the soil content of gypsum to the limits of 250-350 g/kg^{-1} leads to improving the physical properties of the soil such as bulk density and its reflection in the total porosity of the soil, which increases the soil's ability to hold water, which increases the values of the lowest specific range. from water . The LLWR values decrease when the soil content of clay increases, as the high content of clay results in a lower bulk density and greater water retention. As a result, the LLWR values decrease [5]. It is also clear from the figure that with an increase in the gypsum content in the soil to 440 g/kg^{-1}, the values of to$LLWR_{10}$ and $LLWR_{33}$ decreased to 0.115 and 0.069 $cm^{-3}cm^{-3}$, respectively. Figure 3 also shows that the values of $LLWR_{10}$ and LLWR33 were close at low gypsum levels, while the difference between them increased with increasing soil content of gypsum, as 3 reached 0.023, 0.014, and 0.057 $cm^{-3}cm^{-3}$ for gypsum levels of 60, 100, and 150 g kg^{-1}, respectively. While it reached 0.093 and 0.070 $cm^{-3}cm^{-3}$, respectively, at a gypsum content of 200 g kg^{-1}, and the increase in the difference continued for the rest of the gypsum levels in the study soils. Limit values for $LlWR_{33}$ were lower than $LLWR_{10}$ and compatible with Plant Ready Water values PAW_{10} and PAW_{33} (Figure 2) The values of PAW_{10} and $LLWR_{10}$ can be adopted when the soil content of gypsum is more than 200 g/kg^{-1}. The reason for this may be due to the fact that increasing the soil content of gypsum to the limits of 200 g/kg^{-1} leads to a difference in the distribution of pore sizes in the soil, and thus the ability of the soil to hold water decreases when the tensile strength is increased from 10 to 33 kPa It was reached in Figure 1). Gypsum soils behave like sandy soils and their ability to (retain water decreases due to their low porosity on the one hand and their low clay content on the other hand [20].

3.4. The Impact of Soil Gypsum Content Material at the Incorporated Water Capability (IWC)

Integrated water capacity (Integrated Water Capacity, IWC) is a latest concept that considers the willpower of water availability for cultivation by using progressively affecting the bodily residences of soil and water uptake by plants. Figure 4 shows the impact of the soil content material of gypsum at the IWC values, as it is clear that the correlation is superb between the soil of gypsum and the IWC, because the IWC values elevated inside the soil content material of gypsum and reached 0.203 $cm^{-3}cm^{-3}$ when the soil content of gypsum was 440 g/kg^{-1} In the soil inside the soil became 0.006 cm $^{-3}$cm $^{-3}$ within the soil content material of gypsum^{-1} .100 g kg ^{1}. The value of IWC depends on the absolute differential water capacity C(h), which represents the slope of the soil moisture characterization curve and the factor of multiplying the functions of the effect of the weight and physical characteristics of the soil determining plant growth in terms of moisture tension *w*(h) (Equation 3), so the decrease in the IWC values with an increase in soil content of gypsum due to the increase in the slope of the soil moisture description curve on the one hand and the increase in the total water capacity and soil aeration conditions on the other hand, and it is noted that the IWC values are associated with PAW_{10} as they were close, which represents one of the factors determinants of plant growth that provide suitable moisture and aeration conditions for plants [8].

3.5. The Impact of Gypsum Soil on the Critical Power Values of Water to be had to Flowers [EI (PAW)

Figure 5. shows the effect of the soil content of gypsum on the values of the integrated energy of water available to the plant, $EI_{(paw)}$, at tensile strengths of 10 kPa $El_{(paw10)}$ and 33 kPa $El_{(paw33)}$, as it is noted that these values decrease with the increase in the soil content of gypsum, reaching 168.92 and 0269.9 J at the soil content of gypsum 440 g kg^{-1}Respectively, while these values increased with the decrease in the soil content of gypsum, reaching 233.47 and 323.52 J when the soil content of gypsum was 60 g/kg^{-1}, respectively. It is clear from Figure 5 that the values of the integrated energy of water available to the plant at $El_{(paw10)}$ were less than $El_{(paw33)}$ with the difference in the soil content of gypsum. It is

noted that the difference between the values of $El_{(paw10)}$ and the values of $El_{(paw10)}$ increases with the increase in the gypsum content in the soil, as it increased from 90.05 to 100.98 joules with the increase in the soil content of gypsum. From 60 to 440 g/kg^{-1}, which indicates that the tensile strength of 10 kPa is better for the plant because it requires less energy to absorb the available water. The $El_{(paw)}$ increases with the increase in bulk density.

Both the tensile $El_{(paw10)}$ and $El_{(paw33)}$ increase with the increase in the clay content. Thus The plant needs more energy to absorb the available water from the clay soil than the sandy one [12]. It turns out that increasing the clay content on EI $_{(PAW)}$ requires more energy to absorb the available water from soft soil compared to coarse soil [1]. A high negative correlation was found between EI $_{(PAW10)}$ or EI $_{(PAW33)}$ And the parameter (n) is the form factor of the moisture characterization curve of the van Genuchten equation, which explains the decrease in the values of $EI_{(PAW)}$ with an increase in the slope of the soil moisture characterization curve [12].

3.6. Effect of Soil Gypsum Content Material on the Integrality Values of the Bottom Limiting Range of Water [EI (LLWR)]

Figure 6 indicates the effect of the soil content material of gypsum at the crucial power values of the lowest proscribing variety of water EI $_{(LLWR)}$ at tensile strengths of 10 kPa EI $_{(LLWR10)}$ and 33 kPa EI $_{(LLWR33)}$, as those values decrease with the growth inside the soil percentage of gypsum, achieving 168.92 And 269.90 j at EI $_{(LLWR10)}$ and EI $_{(LLWR33)}$ at the soil content of gypsum 440 g/kg^{-1}, respectively, while these values increased with a decrease in the gypsum content in the soil, reaching 658.18 J at both stresses EI $_{(LLWR10)}$ and EI $_{(LLWR33)}$ when the soil content of gypsum was 60 g/kg^{-1}. The results also show that the difference between the integrative energy values EI $_{(LLWR10)}$ and EI $_{(LLWR33)}$was high at the levels of gypsum 60, 100 and 150 g kg^{-1}, reaching 658.18, 800.79 and 367.61 joules, respectively, then decreased to 168.92 and 269.90 joules when the soil content of gypsum 440 g. kg^{-1}. This can be attributed to the rule

Large soil pore sizes at the expense of small soil pores by increasing the proportion of gypsum from 200 to 440 g kg $^{-1}$. It is also clear from Figure 6 that the values of EI $_{(LLWR33)}$ are greater than EI $_{(LLWR10)}$ by about 100.98 at a gypsum content of 440, which indicates that tensile strength of 10 kPa is better for plants because it requires less energy to absorb the available water. $EI_{(LLWR)}$increases with increasing bulk density, so the decrease in the values of $EI_{(LLWR)}$with an increase in the soil content of gypsum is due to an increase in the slope of the moisture characterization curve [12].

3.7. The Impact of Soil Content Material of Gypsum at the Integrality of the Included Water Capability EI (IWC)

Figure 7 shows the impact of soil content material of gypsum at the vital values of the integrated water ability EI $_{(ICW)}$, as it is clear that the relationship is terrible between the soil content material of gypsum integrated water capacity EI $_{(ICW)}$values of EI $_{(ICW}$) the proportion of soil in the soil content material, 92.811 joules in the soil content of.440 g kg^{-1}, while these values increased with the decrease of the soil content of gypsum to reach 836.02 joules at the soil content of gypsum is 60 g/kg^{-1}.

Figure 8 shows that the effect of the soil content of gypsum on decreasing the values of EI $_{(ICW)}$, a gradual decrease in water ready soil due to the resistance of the soil to penetration in the dry range [21]. With an increase in the content of gypsum in the soil from 150 to 440 g kg^{-1}, the results are consistent with figure 7 Because with an increase in IWC, the integrated water content confined between the moisture tension at saturation of 0.1 and 1500 kPa increases, which means with an increase in the soil content of gypsum due to the increase in the slope of the soil moisture description curve in one direction and the increase in capacity Total water and soil aeration conditions, on the other hand [8].

Table 2. The studied percentages of gypsum with PAW, LLWR, IWC.

	Gypsiferous soil	PAW_{100}	PAW_{330}	$LLWR_{100}$	$LLWR_{330}$	IWC
G1	60	0.0848	0.0597	0.0239	0.0239	0.0107
G2	100	0.0759	0.0523	0.0142	0.0142	0.0063
G3	150	0.0881	0.059	0.0478	0.0478	0.0205
G4	200	0.1063	0.07	0.0934	0.07	0.066
G5	250	0.1289	0.0842	0.1289	0.0842	0.1194
G6	350	0.1342	0.0817	0.1342	0.0817	0.1912
G7	440	0.1146	0.0686	0.1146	0.0686	0.2034

Table 3. The studied percentages of gypsum with $EI_{(PAW)}$, $EI_{(LLWR)}$ $EI_{(IWC)}$.

	Gypsiferous soil	$EI_{(PAW100)}$	$EI_{(PAW330)}$	$EI_{(LLWR100)}$	$EI_{(LLWR330)}$	$EI_{(IWC)}$
G1	60	233.47	323.52	658.18	658.18	836.02
G2	100	224.35	314.05	800.79	800.79	956.16
G3	150	211.37	306.12	367.61	367.61	558.07
G4	200	205.27	301.63	231.82	301.63	302.76
G5	250	202.18	299.45	202.18	299.45	208.32
G6	350	174.67	274.93	174.67	274.93	119.02
G7	440	168.92	269.9	168.92	269.9	92.811

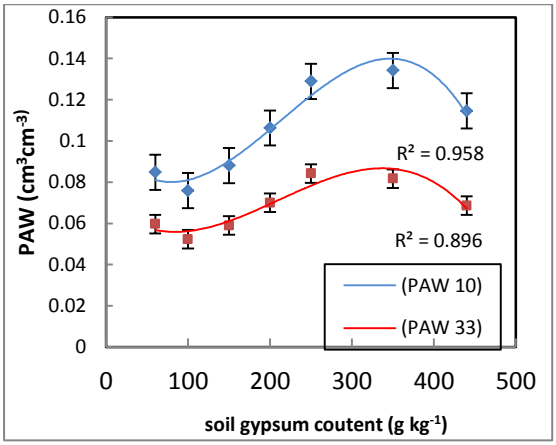

Figure 2. Effect of soil content of gypsum on values PAW_{100} and PAW_{330}.

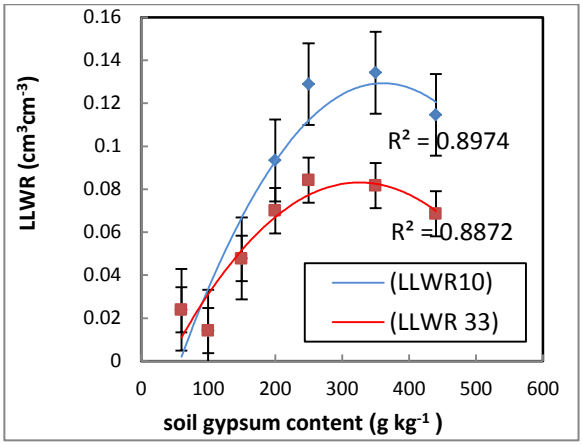

Figure 3. Effect of soil content of gypsum on values $LLWR_{100}$ and $LLWR_{330}$.

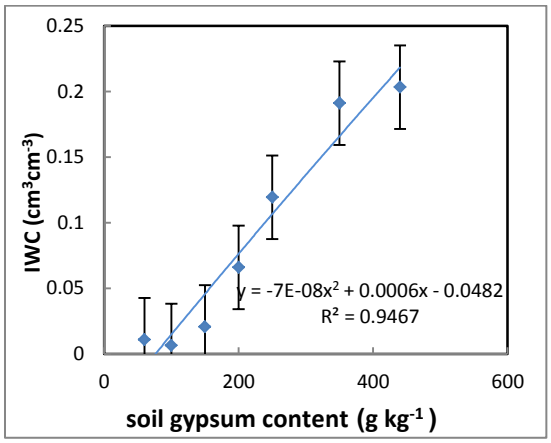

Figure 4. Effect of soil content of gypsum on values (IWC).

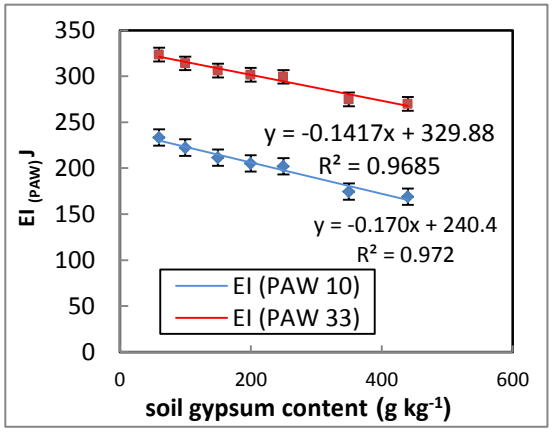

Figure 5. Effect of soil content of gypsum on values (EI_{PAW}).

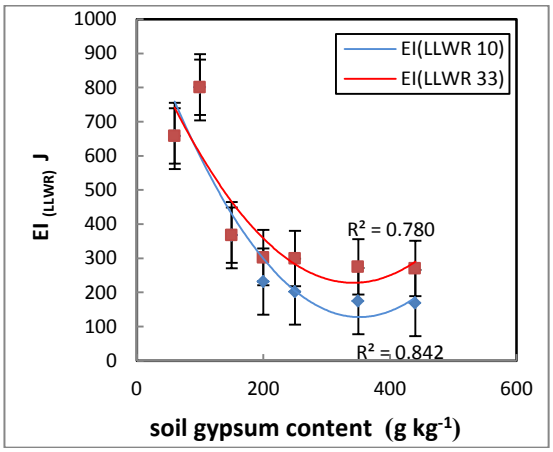

Figure 6. Effect of soil content of gypsum on values EI $_{LLWR}$.

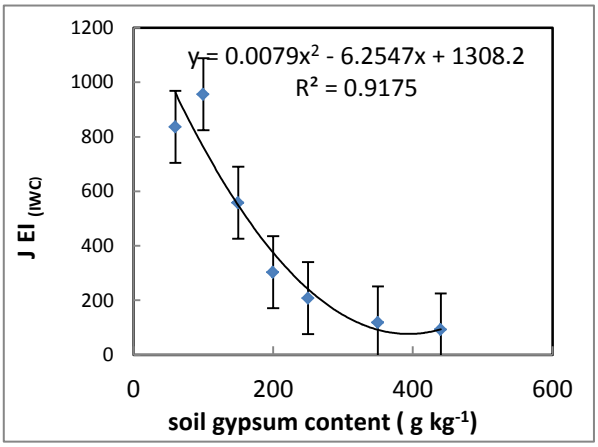

Figure 7. Effect of soil content of gypsum on values EI $_{IWC}$.

Conclusions

10% The effective pore diameter increased with the increase in the proportion of gypsum in the soil. It reached 10% for the soil model with low gypsum content (G1), and ranged between 10-15% for soils with low gypsum content (G2-G7) , The water available to plants increased (PAW_{10}) with an increase in the soil content of gypsum from G1 to G6 as it reached 8-12% compared to (PAW_{33}), The water available to plants increased (PAW_{10}) with an increase in the soil content of gypsum from G1 to G6 as it reached 8-12% compared to (PAW_{33}) , The minimum specific range for water ($LLWR_{10}$) increased compared to ($LLWR_{33}$) with an increase in the soil content of gypsum, as it reached 2.52% for the soil model G1 and 13-8% for the soil models G6 respectively, The integral water capacity (IWC) increases with dullness .

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A comparative Study Between the Performance of Emitters Types, Operating Pressure Levels, and Response of Two Classes of Cucumber under Drip Irrigation System

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Abstract. The study was conducted at the Al-Hawija gardening, in AL Hawija district. Which is located southwest of Kirkuk Governorate, for the summer season 7/18/2020. The study included the evaluation of the drip irrigation system with four different types of drip irrigation system emitters (T-Tape - GR - Turbo - Spiral). Three levels of operating pressure (1.5 - 1 - 0.5) bar, and two varieties of cucumber crop. The experiment designed according to the randomized complete block design (R.C.B.D). The differences between the averages tested according to the Duncan test to extract the significant differences among the averages of the treatments at the probability level 0.05. When evaluating the drip irrigation system the results showed the superiority of the GR emitter in characteristics, coefficient of variation, water addition efficiency, regularity of absolute field emission, and water consumption. It also became clear that the pressure of 0.5 bar had a significant effect on the coefficient of variation and the efficiency of addition. The pressure of 1 bar was superior in the characteristics of uniformity of absolute field emission and water consumption. The 1 bar pressure treatment and the emitter GR were superior to the rest of the treatments of coefficient of variation, water addition efficiency, absolute field emission uniformity, and water consumption in relation to the characteristics related to system evaluation, plant height, and total yield in relation to plant traits. The results showed that the cultivar Omiga was significantly superior to the cultivar Abu Zagheib in the characteristics of plant height and total yield .

Keywords. Drip Irrigation System, Coefficient of variation, Water addition efficiency, Absolute uniformity of field emission, Estimation of water consumption, Total yield, Plant height.

1. Introduction

The drip irrigation system, when compared to other irrigation methods, is one of the greatest that the world has utilized because of its excellent quality and consistency. This method uses a network of pipes to carry water to the field, which is then transferred to the field using emitters. Despite the benefits of drip irrigation, the technique has a number of flaws, including the impact of soil type and distribution. To meet the objectives of employing a drip irrigation system, it must be correctly built and managed, with rates and locations of water supply to the root zone that are appropriate for the

crops' needs, because it feeds the plant with water in the root area, the drip irrigation system is one of the most efficient techniques of irrigating crops, with an efficiency of up to 95%. As a result, water losses due to deep penetration, surface run-off, and evaporation are exceeded. When the drip drain variance is less than 7%, it's acceptable; when it's more than 11%, it's unsuitable[1]. The uniformity of water distribution for drip irrigation networks is the result of a group of factors, including the operating pressure available from the pump, the pump drain, the pressure differences resulting from friction in the water-carrying and distributing pipes, the diameter and length of the pipe, as well as the topography of the land, the type of drips, their blockages, and their manufacturing variation [2]. The drip irrigation system is considered an effective factor in displacing the accumulated amounts of salinity in the root zone of the soil, in which the salinity rate increases, and thus reduces the water effort used in these lands due to the germination and growth of the cultivated plants that are irrigated with this system.

Several approaches are utilized to improve the crop, including managing light durations and temperatures by selecting planting dates, as well as selecting adequate and current irrigation systems. Vegetable crops are known for their high water consumption, thus using a drip irrigation system to irrigate them resulted in a reduction in water usage. The cucumber plant belongs to the Cucurbitaceae family. It is one of the groups of the melon plant, Cucumis melo L. which is a monoecious plant that carries male and female flowers separately on the same plant. It is considered one of the important summer vegetables in Iraq whose fruits are consumed fresh or pickled [3]. Accordingly, the aim of the study is:

- Testing and evaluating the drip irrigation system and thus leading to the best operating pressure.
- Reaching the best evaluation of the types of emitters.
- Reaching the best class that can be planted from the studied classes under the conditions of this experiment.

2. Materials and Methods

The experiment was carried out at the Hawija horticultural station affiliated to Kirkuk Agriculture Directorate / Ministry of Agriculture, in Hawija district, which is located in the southwest of Kirkuk governorate, 66 km from the center of the governorate. The soil texture was silty sand.

The drip irrigation system network was installed in the field according to the experimental design and the factors related to the system.

- Emitters: Four types of emitters (T-Tape, GR, Turbo and Spiral) were used.
- Operating pressure: The first line: (1.5 bar) The second line: (1 bar) The third line: (0.5 bar).

2.1. Studied Properties

2.1.1. Properties Related to the Evaluation of the Drip Irrigation System

2.1.1.1. Coefficient of Variation (CV %), [4]

$$CV(\%)=SD/qa \quad (1)$$

CV = coefficient of variation (%).
SD =standard deviation of drains (L h^{-1})
qa = general average emitter drains (L h^{-1}).

2.1.1.2. Water Addition Efficiency (%) EU [5]

$$EU(\%)=100\ [1-(1.27\times CV/\sqrt{n})]\times(qn/qa) \quad (2)$$

EU = Emission Uniformity (%).
CV = coefficient of variation (%).
qn = average of least discharges for 1/4 of the total number of emitters tested (L h-1).
qa = general average emitter drain (L h^{-1}).

n =number of emitters.

2.1.1.3. Absolute Uniformity of Field Emission F.EUa (%) [6]

$$F.EUa(\%)=50[(qn⁄qa)/(qa⁄qx)] \quad (3)$$

F.EUa = Absolute Field Emission Uniformity %.
qx = average of the highest drains for 1/8 of the total number of emitters tested (L h^{-1}).

2.1.1.4. Estimation of Water Consumption (mm) [7]

$$Eta= (P + Ir) - \Delta s \quad (4)$$

Eta = Water consumption (mm).
P = Amount of rain mm.
Ir = Amount of irrigation water in grams.
Δ s = Difference in soil moisture in grams.

2.1.2. Properties Related to Plants

2.1.2.1. Plant Height (cm)
The length of the plant was measured using a tape measure for five plants from each experimental unit, and the average was calculated.

2.1.2.2. Total Yield (Mgs/ha)
It was calculated from the yield of plants in the experimental unit, then the yield of the experimental unit and the yield of one hectare was calculated according to the following two equations [8]:

Experimental unit yield = yield per plant x number of selected plants

Total yield in hectares = {(Experimental unit quotient x 10000)/ (Experimental unit area)}

where 10,000 = the cultivated hectare area in square meters.

3. Results and Discussion
Discussion of the studied properties of the drip irrigation system.

3.1. Coefficient of Variation CV %
Table (1) showed significant differences according to the statistical analysis and its impact on this feature among the average emitters and operational pressures, where the emitter GR achieved the least difference and amounted to 0.058, which falls within the medium range of drip efficiency. The Turbo emitter on the other hand recorded the highest difference of 0.148, which falls within the weak range for drip efficiency. At different levels of operational pressure, the operating pressure 0.5 bar achieved the lowest difference and amounted to 0.071, which falls within the lower-than-average rank, while the operating pressure 1.5 recorded the highest difference and amounted to 0.138, which falls within the weak range of drip efficiency.
The emitter that achieved GR with the operating pressure of 1 bar had the lowest difference of 40.05, which was within the average range of the drip efficiency in Table (2), and this was reflected positively on the plant characteristics. The Turbo emitter recorded with the operational pressure 1.5 bar had the highest difference of 40.05, which was within the average range of the drip efficiency in Table (1), and this was reflected positively on the plant characteristics. Even between two similar emitters, changes in manufacturing, operating pressure, emitter obstruction, emitter consumption, friction, change in irrigation water temperature, and emitter sensitivity all lead to a change in flow rate [10]. This is in agreement with what was indicated by [9,11].

Table 1. The efficiency of the emitter according to the differential value of the coefficient of variation [9] for comparison.

Emitter efficiency	Coefficient of variation CV
Excellent	0.05< C.V
Middle	0.07 > CV > 0.05
Less than middle	0.11 > C.V < 0.07
Weak	0.15 > C.V > 0.11
Unacceptable	0.15 > C.V

Table 2. The effect of the types of emitters, the operating pressure and the interaction between them on the properties of CV %.

Levels of emitter types (A)	Levels of operating pressure of drip system(bar) (B)			Averages of emitters
	0.5	1	1.5	
GR	B 0.056	B 0.054	B 0.063	B 0.058
Spiral	B 0.075	B 0.070	B 0.072	B 0.072
T-Tape	B 0.067	A-B 0.168	A-B 0.171	A-B 0.135
Turbo	B 0.086	B 0.112	A 0.246	A 0.148
Average of pressure	0.071 B	A-B 0.101	A 0.138	

According to Duncan's Test at a probability level of 5%, averages that share comparable alphabetical letters show no significant differences.
The lower value is the best value.

3.2. Design Emission Uniformity (%) EU

According to the statistical data, there are considerable disparities between the averages and their influence on this feature, as shown in Table (3). Significant variances exist across emitter types, with the GR emitter achieving the maximum efficiency of 97.22 percent, while the T-Tape emitter achieving the lowest water addition efficiency of 95.64 percent. 97.10 percent. With a water addition efficiency of 96.04 percent, the operating pressure 1.5 had the lowest water addition efficiency. When overlapping the types of emitters and the levels of operational pressure in the same table, we see that the GR emitter with a 1 bar operating pressure obtained the best combination in water addition efficiency, at 98.68 percent. The T-Tape emitter with an operating pressure of 1 bar recorded the lowest water addition efficiency and amounted to 92.46%, and the reason for these differences among the values, low irrigation rates were more effective in increasing irrigation efficiencies than high irrigation rates. The results indicate the extent of the efficiency of the emitter in the good distribution of irrigation water and its positive impact on the studied characteristics and in increasing the yield and its qualitative characteristics. This is consistent with what was stated by [11-13].

Table 3. The effect of emitter types and operating pressure and the interaction between them on the water addition efficiency, % EU.

Levels of emitter types (A)	Levels of operating pressure of drip system(bar) (B)			Averages of emitters
	0.5	1	1.5	
GR	A-B 96.67	A 98.68	B-C 96.31	A 97.22
Spiral	A-B 97.74	A-B 96.92	A-B 96.30	A 96.99
T-Tape	A-B 97.50	C 92.46	A-B 96.97	B 95.64
Turbo	B-C 96.47	A 98.67	A-C 94.59	A-B 96.58
Average of pressure	97.10	96.68	96.04	

According to Duncan's test at a probability level of 5%, averages that share comparable alphabetical letters show no significant differences.

3.3. Absolute Uniformity of Field Emission F.EUa (%)

According to the statistical analysis, Table (4) shows that there are significant differences between the averages and their impact on this feature, with the emitter GR achieving the highest absolute field emission regularity of 98.86 percent and the Turbo emitter achieving the lowest absolute field emission regularity of 95.82 percent, and at various operating pressures. There are no notable variations between the operating pressures, with the operating pressure of 1 bar achieving the maximum absolute field emission uniformity of 98.02 percent and the operating pressure of 1.5 bar achieving the lowest absolute field emission uniformity of 96.57 percent. Through the same table, the overlap between the types of emitters and the levels of operational pressure had an oscillatory effect, whereby the GR emitter, in combination with the operating pressure of 1 bar, achieved the highest uniformity of absolute field emission F.EU.a%, which amounted to 98.93%, and the Turbo emitter with the operating pressure recorded 1.5 bar less. The uniformity of absolute field emission F.EU.a% amounted to 94.64%, where we note that all the averages fall within the excellent range according to Table (5) as they are more comprehensive than F.EU%. The reason is due to the increase in the operating pressure in the drip irrigation system to a certain extent which leads to the regularity of the water leaving the droplets in the field. The higher the values of the emission regularity, the more uniform the water distribution in the field and close to the ideal state [14]. This is consistent with [4,11,15]

Table 4. Uniformity of absolute field emission F.EUa% (standard) according to the American Standard Recommendations Society of Agricultural Engineers [6].

Values	F.EU%	% F.EU.a
Excellent	More than 90%	% 100_94
Very good	% 90_80	% 87_81
Good	% 80_70	% 75_68
Accepted	Less than70 %	% 56_68

Table 5. The effect of drip types, operating pressure and the interaction between them on the uniformity of the absolute field emission FEUa %.

Levels of emitter types (A)	Levels of operating pressure of drip system(bar) (B)			Averages of emitters
	0.5	1	1.5	
GR	A-B 98.10	A 99.93	A 98.55	A 98.86
Spiral	A-B 97.97	A 99.15	A-B 96.79	A-B 97.97
T-Tape	A-B 97.75	A-B 96.49	A-B 96.28	A-B 96.84
Turbo	A-B 96.30	A-B 96.51	B 94.64	B 95.82
Average of pressure	97.53	98.02	96.57	

The averages that share similar alphabetical letters have no significant differences between them according to Duncan's test at the probability level of 5%.

3.4. Measurement of Water Consumption (mm)

According to the statistical analysis, there are substantial variations between the averages and their influence on this characteristic, where the spiral emitter attained the maximum water consumption, which was 39.33 mm. The emitter T-Tape used the least quantity of water, at a total of 29 mm. Water usage was highest at 1 bar (37 mm), and lowest at 0.5 bar (28.50 mm). When overlaying the kinds of emitters and the levels of operating pressure in the same table, the treatment of the spiral emitter with the operating pressure 1 bar recorded the greatest water consumption, which was 46 mm. The T-Tape treatment with a 0.5 bar working pressure used the least amount of water and resulted in a water consumption of 25 mm. Despite the fact that these two treatments had no influence on the rest of the characteristics, the emitter GR treatment with a 1 bar operating pressure attained 33 mm was consistent with the other plant features as well as the drip properties. The reason is that the moisture content that is above the plant's need will cause fungal diseases and root rot and vice versa if less content than the plant needs, plant growth will not be optimal.

Table 6. The effect of drip types and operating pressure and the interaction between them on the characteristic of water consumption ET (mm).

Levels of emitter types (A)	Levels of operating pressure of drip system(bar) (B)			Averages of emitters
	0.5	1	1.5	
GR	C 28.00	B-D 33.00	A-B 41.50	A-B 34.17
Spiral	C 30.00	A 46.00	B-D 42.00	A 39.33
T-Tape	C 25.00	D 29.50	B-D 32.50	B 29.00
Turbo	C-D 31.00	A-C 40.00	C-D 31.00	A-B 34.00
Average of pressure	B 28.50	A 37.13	36.75 A	

The averages that share similar alphabetical letters have no significant differences between them according to Duncan's test at the probability level of 5%

3.5. Discussing the Results of the Studied Features Related to the Cultivated Crop Varieties

3.5.1. Average Plant Height (cm plant^{-1})

The results in Table (7) showed that this characteristic was significantly affected by the operational pressure treatment, as the operating pressure 1 bar achieved the highest average height of the plant, which reached 116.50 cm plant^{-1} compared to the operational pressure of 0.5 bar, which recorded the lowest plant height and reached 97.32 cm. Plant-1. As for the different classes, there were no significant differences, as Omega class achieved the highest plant height, which reached 108.72 cm plant^{-1} compared to the minimum plant height of 105.80 cm plant^{-1}, which was recorded by Abu Zughaib class. The reason may be due to the different genotypes of the classes used in the study, which caused the difference in their response as well as the treatment of the species of the pips. We note that there are significant differences as the treatment of the spiral pips achieved significantly, reaching 116.98 cm plant^{-1}, while the lowest length was recorded. The plant has the treatment of the emitter T-Tape 93.62 cm plant^{-1}, where the drain of water has a great role in providing the moisture necessary for the dissolution of major elements in the soil such as nitrogen and other elements. This could affect the readiness of these elements for the plant and their subsequent use [16].

Through the same table, the triple interference coefficients show significant differences between the treatments in their effect on this property, as the operating pressure treatment 1.5 bar, GR and Omega class achieved the highest plant height, which reached 128.43 cm. Plant-1 compared to the treatment of operational pressure 0.5 bar, T-Tape and Abu Zagheib class, which recorded the lowest plant height, which was 83.20 cm. Plant-1. The reason for the plant's height may be due to the different genotypes of the varieties used in the study, which caused the difference in its response. This is consistent with what was found by [17] for the cucumber plant.

3.5.2. Total Yield (Mg ha^{-1})

It is clear from the results of Table (8) that there are significant differences between the levels of operational pressure in the characteristic of the total yield Mg h^{-1}. The operating pressure of 1 bar achieved the highest rate of total yield, which amounted to 29.53 Mg h^{-1}, compared to the operating pressure of 0.5 bar, which recorded the lowest rate of total yield of 21.28 Mg h^{-1}. As for the classes, Omega was significantly superior to the other classes, as it achieved 28.14 Mg h^{-1}, while the Abu Zagheib class recorded the lowest weight of the total yield, which amounted to 22.71 Mg h^{-1}. This characteristic was also affected by the types of emitters, as the spiral emitter achieved the largest weight of the total yield and amounted to 28.36 Mg h^{-1} over other emitters that recorded the lowest total yield of the T-Tape, which reached 21.38 Mgs.ha^{-1}.

It is noted from the results of the same table in the triple overlap coefficients that there are significant differences between the treatments, as the operating pressure treatment achieved 1 bar, the drip spiral. Omega variety, amounted to 40.36 Mg h^{-1}, while the operational pressure treatment bar 0.5, T-Tape and Omega variety recorded the lowest weight of the total yield as it reached 17.42 Mg h^{-1}. The reason for this may be due to the increase in moisture content, which in turn affects the physiological processes of the plant and by increasing the humidity content, the wet area increases. This was noticed by the depth of wetting in the stream, which affects the yield of the crop.

Table 7. The effect of drip irrigation water pressure and types of emitters for two classes of cucumber and the interaction between them on plant height (cm plant^{-1}).

Levels of plant type (A)	Levels of pressure (bar) (B)	Levels of emitter types (C)				Interaction (A+B)	Averages of plant Class (A)
		GR	Spiral	T-tape	Turbo		
Abu Zaghaib	0.5 bar (b1)	100.48 C-H	106.33B-G	83.20 C	E-G 94.13	C 96.04	A 105.80
	1bar (b2)	120.53 A-C	123.18A-B	101.80 C-G	118.09A-D	A 115.90	
	1.5 bar (b3)	117.33 A-E	117.08 A-D	92.41 G-H	E-G 95.00	B-C 105.46	
Omega	0.5 bar (b1)	102.79C-I	108.97 A-F	86.97 F-I	E-F 95.72	C 98.61	A 108.72
	1bar (b2)	122.81 A-B	126.79A	102.48 C-F	A- 116.28 E	A 117.09	
	1.5 bar (b3)	128.43 A-D	119.51 A-C	94.84 E-F	99.00D-F	A-C 110.45	
Interaction (A*C)	Abu-zaghaibA-(a1)	a-b 112.78	A 115.53	C 92.47	C 102.41	Averages of pressure (B)	
	Omega (a2)	A 118.01	A 118.42	C 94.76	B-D 103.67		
Interaction (A*B)	0.5 bar (b1)	C-D 101.64	B-C 107.65	E 85.09	D-F 94.93	C 97.32	
	1bar (b2)	A 121.67	a 24.99	C-D 102.14	ĺAB117.19	A 16.50	
	1.5 bar (b3)	A 122.88	A--b118.30	D-E 93.63	C-E 97.00	B 107.95	
Averages of emitter types (C)		A 115.40	A116.98	C 93.62	C 103.04	B103.04	

The averages that share similar alphabetical letters have no significant differences between them according to Duncan's test at the probability level of 5%.

Table 8. The effect of drip irrigation water pressure, types of emitters and two classes of cucumber and the interaction between them on the weight of the total yield (Mg h^{-1}).

Levels of plant type (A)	Levels of pressure (bar) (B)	Levels of emitter types (C)				Interaction (A+B)	Averages of plant Class (A)
		GR	Spiral	T-tape	Turbo		
Abu Zaghaib	0.5 bar (b1)	19.69 E-F	20.48F-G	18.17C-H	20.86 E-I	C 19.80	B 22.71
	1bar (b2)	30.24 C-D	25.70 E-G	24.10 E-F	22.76 E-F	B 5.70	
	1.5 bar (b3)	22.64 E-F	F- 21.05 G	24.73 D-H	22.04 G-H	c 2.62	
Omega	0.5 bar (b1)	20.32 E-G	29.42B-F	F 17.42	23.91 D-F	C 22.77	A 28.14
	1bar (b2)	39.52 A	Aĺ40.36	21.34 E-G	32.19 B-C	A 3.35	
	1.5 bar (b3)	28.62 B-E	33.15B	-22.54 E-F	28.88 B-C	B 28.30	
Interaction (A*C)	Abu-ĺzaghaib (a1)	C 24.19	C 22.41	C 22.33	C 21.89	Averages of pressure (B)	
	(a2) Omega	B 29.49	A 34.31	C 20.43	B 28.33		
Interaction (A*B)	0.5 bar (b1)	C-E-20.01	B-C 24.95	E 17.80	C-D 22.39	C 21.28	

Levels of pressure (bar) (B)	Levels of emitter types (C)				Interaction (A+B)
	GR	Spiral	T-tape	Turbo	
1bar (b2)	A 34.88	A 33.03	C-D 22.72	B 27.48	A 29.53
1.5 bar (b3)	B-C 25.63	B-C 27.10	B-D 23.64	B-C 25.46	B 25.46
Averages of emitter types (C)	A-B 26.84	A 28.36	C 21.38	B 25.11	B103.04

The averages that share similar alphabetic letters have no significant differences between them according to Duncan's test at the 5% probability level.

Conclusions

- The best emitter was of the GR type, which had the greatest results in many of the researched characteristics related to a better drip irrigation system (coefficient of variation, efficiency of water addition, regularity of absolute field emission and water consumption)
- Through interference, the operating pressure of 1 bar had a significant effect on vegetative development and yield, as well as most of the features of the drip irrigation system.
- In terms of vegetative growth and yield, Omega was superior in every way.
- The triple interaction between the operating pressure 1 bar and the GR and Spiral dots with Omega class gave the highest values for the yield trait.

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Impact of Poly-γ-Glutamic Acid on the Growth and Yield of Corn (*Zea maize* L.) under Partial Drip Irrigation in a Gypsiferous Soil

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Abstract. The alternate partial drying method of the root zone is a modern irrigation method to save the amount of irrigation water. A field experiment was conducted to grow maize under the drip irrigation system conventional irrigation(CI) ,alternate partial root-zone drying (APRD) and the use of polymer levels of (0, 15, 30, 60 and 120) kg. ha^{-1} in a gypsiferous soil. The effect of the alternate partial drying method of the root zone and the levels of Poly-γ-glutamic acid (γ-PGA) addition on the growth and yield of maize plants and the water consumption efficiency were studied. The CI excelled in (plant dry shoot, plant height, and root dry weight) for the three stages: Jointing, Booting, and Maturing. The APRD method was superior in (seed weight, 500 seed weight, and water use efficiency) with an increase rate of (0.5, 0.67 and 17.33) % respectively. As for the addition levels of the polymer, they achieved a significant increase with the increase in the amount of addition in all the studied properties. As for the effect of interaction between the factors, the treatment (I_2PG_4) was the best treatment compared with the control treatment (I_1PG_0) in most of the studied traits. Except for the trait (weight of seeds, weight of 500 seeds, water use efficiency).

Keywords. Water, Gypsiferous Soil, Zea maize L.

1. Introduction

Water is the main determinant of agricultural production in arid and semi-arid regions, and with the scarcity of water resources, the need to pay attention to the optimal use of water and increase the economic value of each unit of water increases. The exacerbation of the problem of water scarcity prompted researchers to adopt many practices that would rationalize water consumption, such as incomplete irrigation, or reducing some irrigations during certain stages of plant growth that do not cause damage to the yield, [1], or that the economic value of the yield decrease is much less than the economic value achieved from Rationalizing the consumption of water that can be used in cultivating new arable lands, but the water share was not secured for it, and therefore the productivity of the water unit achieved will be higher than that of traditional irrigation methods. The researchers recently adopted another method in rationalizing water consumption and raising the efficiency of water use,

which is the use of the partial irrigation technique of the plant's root system (APRD), [2,3] which is one of these modern methods that were proposed to rationalize the consumption of irrigation water, which was developed through irrigation. Incomplete, and the mechanism of this method depends on adding water to one half of the root system in the first irrigation, and then adding it to the other half in the next irrigation [4,3]. The scarcity of irrigation water and soil degradation are two major factors that affect agricultural production, especially in dry areas and semi-arid [5]. Extreme long-term climatic conditions (such as little or no rainfall and high evaporation) can lead to severe environmental problems, such as desertification of arable land and decreased fresh water resources [6]. Gypsiferous soils are spread in Iraq and constitute more than 20% of the area of Iraq [7], and are characterized by containing different proportions of gypsum, which makes them characterized by poor construction and deterioration of their physical characteristics foremost of which is their poor ability to abrasion [8]. To retain water and nutrients, which negatively affects the water consumption of various crops, and thus affects the growth and yield of these crops alike. In order to increase the ability of the gypsiferous soil to retain water, methods and practices were used on the soil. Examples of this are conservation agriculture (conservation tillage) or adding materials to the soil that increase its ability to retain water, such as adding organic matter [9]. Recently, super-absorbent and water-saving organic polymers have been used and added water-saving superabsorbent polymers, and among these polymers is Poly-γ-glutamic acid, (γ-PGA) which is a promising and environmentally friendly polymer, in addition to its biodegradability, non-toxicity and the ability to absorb water in quantities estimated at thousands of times its own weight, [9,10]. If γ-PGA acid is used in dry areas, it can be likened to a "miniature tank". It is an amino acid and its molecular formula is (C5H9NO4). This polymer has been widely used in the food, medicine, water treatment and agricultural industries Numerous studies have shown that γ-PGA can effectively reduce NO3 -N and NH4 + -N leaching into soil, as well as improve soil water holding capacity (WHC), plant growth and yield increase, plant water stress tolerance and drought resistance [9,11,12]. [13] found that γ-PGA glutamic acid significantly improved the plant uptake of nitrogen (N), phosphorus (P) and potassium (K) and thus increased plant biomass and significantly enhanced the plant's ability to absorb nutrients by enhancing root biomass. While the results of studying γ-PGA under field conditions are still not well understood. Therefore the goal of the presenstady is to evaluate the influence of γ-PGA adding to a gypsifereus soil modification of soil structure and stability on the one hand, and improving the growth yield of corn (.Zea mays L) and weter use efficiency under partial drop root-zon drying.

2. Materials and Methods

2.1. Location and Basic Soil Characteristics

A field experiment was carried out to study the water needs of corn (*Zea mays* L.) under the Conventional irrigation (CI) on both sides of the plant and Alternate Partial Root-zone drying (APRD), during the autumn season 2022 in Al-Alam district, Salahaddin, North of Iraq, located at 34º43′17″ N latitude and 43 º 42′ 5″ E longitude at an elevation of 250 m above mean sea level. The climate of the study area is arid with an average annual rainfall of 150 mm. The rainfall occurs from October to April (rainy season). The study site characterized by a flat topography and the soil sown with grain crops annually. Table 1 shows some of the physical and chemical characteristics of the study site for a depth of (0-0.3) m.

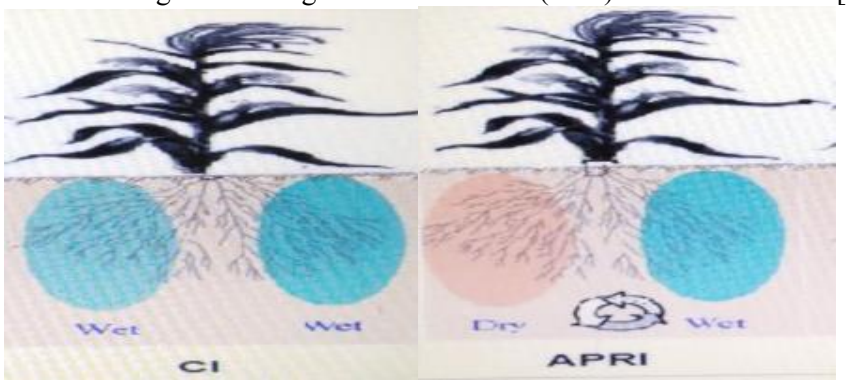
2.2. Field Treatments Preparation

The land was plowed and the soil was smoothed, after that the field was leveled and divided into 4 x 4 m 2 slabs, leaving a 1.0 m interval between one slab and another as a precautionary measure to prevent the transfer of water between the experimental units, as well as using it to lay irrigation pipes. The field surrounded by guard boards to ensure uniform conditions for all transactions.

2.3. Design and experiment parameters

A factorial experiment carried out with split plot designed, with random complete block distribution, with three replications. The experimental coefficients included two factors distributed as follows:

- The main plots: the drip irrigation method (the traditional method of irrigation Conventional irrigation CI on both sides of the plant and the Alternate Partial Root-zone drying APRD). (The plants irrigated through two drip irrigation tubes on both sides of the plant for treatments in which alternate irrigation not applied. As for the alternating irrigation treatments, the plants are irrigated from one side of the root system with the dripper closed on the other side, and in the next irrigation it is irrigated from the side that was not previously irrigated and the dripper is closed. The other and so on alternately throughout the growth period).
- Secondary plots: Poly-γ-glutamic acid (γ-PGA) levels, which are (0, 15, 30, 60 and 120) kg. ha^{-1}. The statistical program SAS was used to analyze the data statistically and compare the averages when testing the least significant difference (LSD) at the level of 5% [14] .



(CI) APRD

2.4. Farming

Yellow maize seeds sown for the fall season on 1/7/2022 by 3-4 seeds in each bore, then the process of grafting and thinning into one plant carried out ten days after emergence. Cultivation is carried out in the experimental unit in the form of lines with a number of five lines, the distance between the lines is (75) cm. In each line there are (16) walls, the distance between the walls is (25) cm. So that the total number of plants in the experimental unit is (80 plants), and after germination, a guard plant is left at both ends of each line, and a line is left planted from the beginning and end of the experimental unit, so that a full collar of guard plants (38 plants) surrounds the experimental unit. Urea fertilizer (46% N) was added at the rate of 200 kg N. ha^{-1} and in two batches: The first batch was added with 100 kg N. ha^{-1} and 150 kg P. ha^{-1} of triple superphosphate fertilizer (21% P) and 120 kg K. ha^{-1} of potassium sulfate fertilizer (43% K) when planting. Fertilizers were added according to the aforementioned doses, in a hole 10 cm away from the planting line, at a depth of 10 cm, and within Wetting area. The second batch of nitrogen fertilizer added 30 days after emergence with irrigation water. Weeding carried out periodically and for all treatments. The yellow corn stem borer, Sesamia cretica L., controlled by using diazinon granular 10% active substance at a rate of 6 kg ha^{-1} inoculated in the heart of the plant for two times, 20 and 40 days after emergence [15] , five plants are taken randomly from each experimental unit during the growth stages of maize plants, namely:

- The first stage (Jointing) after the appearance of four leaves (3-4 weeks after planting).
- The second stage (booting) after the appearance of the male inflorescence (4-5 weeks after the first stage).
- The third stage (Maturing the stage of emergence of heat until full physiological maturity) (35-40 days after the second stage).

The dry matter yield of the vegetative total, the dry weight of the root total for the three stages, and the grain yield for the third stage.

2.5. Drip Irrigation System Installation

A drip irrigation system installed in the field, which consisted of the following parts:

First: The main unit: included (water tank with a capacity of 2500 liters , Pump , filter (disc filter) , Drainage scale , Fertilizer injector and Pressure regulator)

Second: the distribution system: included

- Main pipes: Pipes with a diameter of 2 inches and a length of 175 meters used. They were supplied with water from the source to be distributed to the branch pipes.
- Sub-pipes: with a diameter of 1.5 inches and a length of 150 meters, which in turn deliver water to the field pipes.
- Drip tape pipes: - Pipes with a diameter of 16 mm and equipped with internal drippers, the distance between them is 0.25 m, and a design discharge of 3 liters. 1 hour - for each drip.

2.6. Measuring the Uniformity of Water Distribution in the Field

After installing the irrigation system and before planting the corn crop, an evaluation of the drip irrigation network conducted to measure the uniformity of distribution according to the following steps:

- Four locations were selected along the field pipelines at the beginning of the line, 1/3 and 2/3 of the distance, and the end of the line.
- Cans of suitable size were placed under the drippers, provided that they were at ground level.
- The system was operated at a constant pressure of 100 kilopascals. (This pressure has been proven after several tests to choose the best pressure).
- The test was conducted for five minutes, after which the cans were collected from under the drippers at the same time.
- The volume of the collected water was measured according to the discharge per dripper - 1 liter per hour.
- The average discharge was calculated by dividing the total discharges of the districts by the total number.
- The average discharge for the lowest quarter was calculated by dividing the discharge by its number.
- The homogeneity of the field distribution was measured at the beginning and end of the planting season
- Using the least quarter equation proposed by [16] mentioned below:

$$Eu = (qn / qa) \times 100 \quad (1)$$

while:

Eu = Homogeneity of distribution %.

qn = discharge rate for the lowest quarter (liter-1 hour).

qa = total discharge rate (-1 liters per hour).

2.7. Irrigation

The irrigation process is carried out for the experimental units on the basis of deplesion 50% of the water available to the plant. The quantities of irrigation water and the time required for irrigation were calculated based on measurements of moisture content in the soil using the Gravimetric Method, taking into account the development and growth of roots during the growing season for the purpose of determining the irrigation time and the amount of water. It must be added, as soil samples were taken from the area in which the active roots of the plant spread and according to the development and growth of the roots during the growing season. The soil moisture content of all experimental units was continuously estimated throughout the duration of the experiment.

2.8. Calculating Field and Crop Water use efficiency and Water Productivity

Field water use efficiency (WUEf) was calculated using the following equation [17].

$$WUE_f = Y/WA \quad (2)$$

while:
WUE_f: field water use efficiency (kg m^{-3}).
Y: grain yield (kg).
AW: Amount of water added in the irrigation process (m^3. $season^{-1}$).

2.9. Dry Matter Yield, Grain Yield and Root Dry Weight

The dry matter yield and dry weight of the root system were calculated separately by estimating the moisture content of (5 plant samples) after cutting and drying them in the oven at a temperature of 65 °C for 48 hours. The grain yield was also estimated by harvesting (10 plants) from the two middle lines of each treatment and converting it to kg. ha^{-1} after adjusting the approved moisture percentage, which is 15%.

2.10. Physical and Chemical Measurements of the Study Soil

Samples of depth 0-0.3 m were taken field designated for the study, then the soil samples were air dried and passed through a sieve with a diameter of 2 mm, then the following characteristics were estimated: Finding the size distribution of soil particles using the method [18] , from which the soil texture was extracted. Bulk density was estimated using the cloud method described by [19]. Gypsum was estimated according to the method described by [20].

Table 1. Some physical and chemical properties of field soil before planting.

Measurement	Units	Soil characteristic
Sand	gr .kg^{-1}	474
Silt		349
Clay		177
Soil texture		Sandy lome
Bulk density	Mg m^{-1}	1.37
FC	cm^{-3} . cm^{-3}	0.23
PWP		0.12
water available		0.11

Field capacity at 33 kPa, and permanent wilting point at 1500 kPa soil water tension, respectively.

3. Results and Discussion

3.1. The Effect of Drip Partial Root-Zone Drying and of Poly-γ-Glutamic Acid (γ-PGA) Addition on Corn Growth in a Gypsiferous Soil

Table 2, shows that there is an increase in the dry weight of the corn plant under Conventional irrigation (CI) condition by 18.32, 13.30, and 7.83 % in comparison with Alternate Partial Root-zone drying (APRD) for the growing stages Jointing, Booting, and Maturing, respectively. Table 3 shows that the depth of the irrigation water added in the traditional irrigation method was (93.10, 156.38, and 186.10 mm, while it was 80.69, 137.79, and 171.94 mm for the APRD treatment for the three growing stages (Jointing, Booting, Maturing) respectively without addition of γ-PGA (control). The increase in dry weight recorded under for the traditional irrigation method at the compared to the APRD could be ascribed to the decrease in the depth of water added to the last irrigation method compared with the depth of water added in the CI method. It is observe that there is an inverse relationship between the depth of water added and the level of polymer addition (γ-PGA) Table (3). The values of the depth of water added in the CI method were 435.58, 420.52, 416.71, 410.98, and 398.67 mm , while the recorded values for the APRD method are 390.42, 370.50, 361.27, 340.40, and 322.40 mm, respectively. There is a good relationship between the level of (γ-PGA) addition and the dry weight, Table (2). The higher addition levels of γ-PGA increased the dry weight of the plant for the three growing stages. The increase of the plant dry weight for the addition levels as compared to the control treatment is 9.12, 21.18, 42.35, and 56.57%, and it achieved the level of addition 60 and 120 kg. ha^{-1} give a significant increase for the Jointing stage 2.97, 5.91, 9.01, and 13.94%, and it achieved the level

of addition 120 kg. ha^{-1} significant increase for the Booting stage 7.70, 10.36, 12.37, and 14.93% and achieved the level of addition 120 kg. ha^{-1} significant increase for the maturing stage.

Table 2. The effect of irrigation methods and the amount of addition of poly-beta-glutamic acid and the interaction between them on dry shoot (gm).

	PG I	CK	15 kg/h	30 kg/h	60 kg/h	120 kg/h	Average
	APRD	55.33	57.67	67.67	86.67	91.00	71.67
	CI	69.00	78.00	83.00	90.33	103.67	84.80
(Jointing)	average	62.17	67.84	75.34	88.50	97.34	
	LSD	I = 89.899		PG = 26.174		I*PG = 37.015	
	APRD	498.67	504.00	521.33	539.33	564.67	525.60
	CI	555.33	581.33	595.00	609.67	636.33	595.53
(Booting)	average	527.00	542.67	558.17	574.50	600.50	
	LSD	I = 304.16		PG = 59.798		I*PG = 84.567	
	APRD	845.67	937.67	953.33	975.33	998.00	942.00
	CI	949.33	995.67	1028.00	1041.33	1064.33	1015.73
(Maturing)	average	897.50	966.67	990.67	1008.33	1031.17	
	LSD	I = 229.47		PG =117.37		I*PG = 165.99	

Table 3. Depth of irrigation water (mm).

		CK	15 kg/h	30 kg/h	60 kg/h	120 kg/h	Average
CI	(Jointing)	93.10	91.63	90.44	92.75	91.02	91.79
	(Booting)	156.38	154.19	146.79	141.81	137.38	147.31
	(Maturing)	186.10	174.71	179.48	176.42	170.27	177.40
	Total	435.58	420.52	416.71	410.98	398.67	
APRD	(Jointing)	80.69	80.38	80.13	76.25	73.58	78.20
	(Booting)	137.79	127.25	120.96	117.88	105.73	121.92
	(Maturing	171.94	162.88	160.19	146.27	143.08	156.87
	Total	390.42	370.50	361.27	340.40	322.40	
Percentage to increase the water added	(Jointing)	15.39	14.00	12.87	21.64	23.70	17.52
	(Booting)	13.49	21.17	21.36	20.31	29.93	21.25
	(Maturing)	8.24	7.27	12.04	20.61	19.00	13.43
	Total	11.57	13.50	15.35	20.74	23.66	

As for the effect of the interaction between the factors in the dry shoot , we note from Table 2, shows the first stage (Jointing) that the best value was for the treatment (I_2PG_4) amounted to 103.67 gm, with a significant increase of 78.37 % compared to the treatment (I_1PG_0) that achieved the lowest value It reached 55.33 gm. In the second stage (Booting), the best value was for the treatment (I_2PG_4) amounted to (636.33) gm, with a significant increase of (24.79)% compared to the treatment (I_1PG_0), which achieved the lowest value of (498.67) gm. And the third stage (Maturing) that the best value was for the treatment (I_2PG_4) which amounted to 1064.33 gm and the lowest value was for the treatment (I_1PG_0) which achieved 845.67 gm. The three treatments (I_2PG_4, I_2PG_3 and I_2PG_2) achieved a significant increase of 21.66, 23.20, and 25.92 % compared with the treatment (I_1PG_0) .

3.2. The Effect of Drip Partial Root-Zone Drying and of Poly-γ-Glutamic Acid (γ-PGA) Addition on Plant Height (cm) in a Gypsiferous Soil

Table 4, shows that there is a significant increase in plant height due to the influence of the irrigation methods used, where the (CI) method achieved an increase in plant height by 21.88 % compared to the (APRI) method in the jointing stage. In the two stages, Booting and Maturing, the increase in plant height was small for the (CI) method compared to the (APRI) method.

While there was a difference in the rates of increase in the depth of the added irrigation water, Table 3, we note in the (CI) method, there was an increase in the depth of the added water by 23.70, 21.25 and

13.43 % compared to the (APRD) method for the three jointing stages, Booting and Maturing. respectively . This result is consistent with [21].

As for the effect of the level of addition of (γ-PGA), all levels of addition achieved a significant increase in plant height compared to the treatment (PG_0), and the rates of increase in height were 18.60, 28.90, 35.54 and 50.15 % for the Jointing stage. As for the Booting and Maturing phases, all treatments achieved increased rates compared to the treatment (PG_0), while the treatments PG_4, PG_3 and PG_4 achieved a significant increase compared to the treatment (PG_0). The increase rates were 4.13, 6.89, 8.97 and 12.41 % in the booting stage and 2.82, 6.21, 9.60 and 15.82 % in the maturing stage. The reason for the increase in plant height is due to the increase in the level of (γ-PGA) addition. Adding conditioners to the soil makes it store a larger amount of water and gradually supplies it to the plant roots when needed [22] and that the soil moisture content increases with the increase in the level of addition [23] .

Table 4. The effect of irrigation methods and the amount of addition of Poly-γ-glutamic acid (γ-PGA)and the interaction between them on plant height (cm).

	PG/I	**CK**	**15 kg/h**	**30 kg/h**	**60 kg/h**	**120 kg/h**	**Average**
	APRD	49.00	54.67	55.33	57.67	67.33	56.80
	CI	51.33	64.33	74.00	78.33	83.33	70.26
(Jointing)	average	50.17	59.50	64.67	68.00	75.33	
	LSD	I = 4.8255		PG = 6.2797		I*PG = 8.8809	
	APRD	142.67	148.33	151.67	155.00	161.67	151.87
	CI	148.67	153.67	159.00	162.33	166.00	157.93
(Booting)	average	145.67	151.00	155.34	158.67	163.84	
	LSD	I = 7.2396		PG = 7.1889		I*PG = 10.167	
	APRD	176.67	182.67	191.67	195	203.33	189.87
	CI	178.33	181.67	185	193.67	206.67	189.07
(Maturing)	average	177.50	182.17	188.34	194.34	205.00	
	LSD	I = 9.3081		PG = 8.4057		I*PG = 11.887	

As for the effect of the interaction between the factors on plant height, we note from Table (4) that the best value was for the treatment (I_2PG_4) and the lowest value was for the treatment (I_1PG_0) for the three stages, Jointing, Booting and Maturing, respectively. The transactions (I_1PG_4, I_2PG_1, I_2PG_2, I_2PG_3 and I_2PG_4) achieved a significant increase by 37.40, 31.29, 51.02, 59.86 and 70.06 % for the jointing stage.The treatments (I_1PG_3, I_1PG_4, I_2PG_1, I_2PG_2, I_2PG_3 and I_2PG_4 achieved a significant increase of 9.15, 13.38, 7.75, 11.97, 14.18 and 16.90 % compared with the treatment (I_1PG_0) for the booting stage. The treatments (I_1PG_2, I_1PG_3, I_1PG_4 , I_2PG_3 and I_2PG_4) achieved a significant increase of 8.52, 10.79, 15.21, 9.65 and 17.05 % compared with the treatment (I_1PG_0) for the maturing stage.

3.3. The Effect of Drip Partial Root-Zone Drying and of Poly-γ-Glutamic Acid (γ-PGA) Addition on the Dry Root weight in a Gypsiferous Soil

From Table No. (5), we note that there is a significant increase in the dry weight of the roots for the irrigation treatment (CI) compared with the irrigation method (APRD), as it achieved an increase rate of (10.03, 3.70)% for the two stages, Booting and Maturing. And an increase rate of (6.66) for the Jointing stage. Upon returning to Table 3, we notice an increase in the depth of the irrigation water added in the (CI) method compared to the (APRD) irrigation method, amounting to 17.52, 21.25 and 13.43 % for the jointing, booting and maturing stages, respectively.

Table 5. Effect of irrigation methods and application level of Poly-γ-glutamic acid (γ-PGA) and the interaction between them on dry root weight of the plant (gm).

	PG/I	CK	15 kg/h	30 kg/h	60 kg/h	120 kg/h	Average
	APRD	5.05	5.38	6.35	8.25	8.75	6.76
	CI	5.68	6.57	7.03	7.76	9.02	7.21
(Jointing)	average	5.37	5.98	6.69	8.01	8.89	
	LSD	I = 0.7005		PG = 0.3719		I*PG = 0.526	
	APRD	45.72	47.28	49.6	51.77	54.66	49.81
	CI	50.76	53.28	54.62	56.38	59.02	54.81
(Booting)	average	48.24	50.28	52.11	54.08	56.84	
	LSD	I = 1.6177		PG = 1.5081		I*PG = 2.1328	
	APRD	80.39	87.49	87.81	88.65	90.76	87.02
	CI	87.41	89.19	89.6	90.18	94.8	90.24
(Maturing)	average	83.90	88.34	88.71	89.42	92.78	
	LSD	I = 1.1718		PG = 1.6497		I*PG = 2.333	

As for the effect of the level of addition of (γ-PGA), all levels of addition achieved a significant increase in the dry weight of plant roots compared to the treatment (PG_0) for the three stages, Jointing, Booting and Maturing, and the percentages of increase were 11.36, 24.58, 49.91 and 65.54 % , 65.54%). 4.23 , 8.02 , 12.11 and 17.83 % , and (5.29 , 5.73 , 6.58 and 10.58 % , respectively. As for the effect of interaction between the factors, all treatments achieved a significant increase compared with the treatment (I_1PG_0) , and the highest percentage increase was 78.61, 29.09, and 17.93 % for the three stages Jointing, Booting, and Maturing, respectively.

3.4. The Effect of The Effect of Drip Partial Root-Zone Drying and of Poly-γ-Glutamic Acid (γ-PGA) Addition on the Seed Yield (gm) in a Gypsiferous Soil

From Table 6, we see that the irrigation method (APRD) achieved an increase in the weight of seeds amounted to (0.5)% compared to the (CI) method. The reason for the increase in weight may be due to the fact that the (APRD) method activated the root system of the plant to make maximum use of moisture and nutrients through periods of wetting and mutual drying of the root zone, and this is consistent with what was obtained by [3,,25,26]. And the levels of addition (γ-PGA) had a significant effect on the weight of the seeds, as the treatments (PG_2, PG_3, PG_4) achieved a significant increase of 9.18, 12.27 and 15.92 % compared to the treatment (PG_0). These results are consistent with the results of the researchers [11,12]. As for the effect of the interaction between the factors on the weight of the seeds, the treatments (I_1PG_2, I_1PG_3, I_1PG_4, I_2PG_2, I_2PG_3, I_2PG_4) achieved a significant increase compared to the treatment (I_1PG_0) with an increase

Table 6. Effect of irrigation methods and application level of Poly-γ-glutamic acid (γ-PGA) and the interaction between them on Seed yield (gm).

PG/I	CK	15 kg/h	30 kg/h	60 kg/h	120 kg/h	Average
APRD	1041.67	1088.67	1168.00	1225.67	1269.67	1158.74
CI	1095.00	1122.33	1165.67	1173.67	1207.00	1152.73
average	1068.34	1105.50	1166.84	1199.67	1238.34	
LSD	I = 128.29		PG = 57.453		I*PG = 81.251	

Percentage of 11.91, 12.19, 12.68, 15.94, 17.67 and 21.90 %. Where the treatment (I_1PG_4) achieved the highest value superior to the treatment (I_2PG_4) with a rate of (5.14)% of the weight of the seeds on the one hand, and from the other hand, we find in Table 3, that there is an increase in the depth of the water used for irrigation in Tarifa (CI) compared to (APRD) method, the increase rate was (23.66%), which is enough to irrigate an area of (0.24) hectares by (APRD) method and (0.19) hectares by (CI) method.

3.5. The effect the Effect of the Effect of Drip Partial Root-Zone Drying and of Poly-γ-Glutamic Acid (γ-PGA) Addition on the Weight of 500 Seeds in a Gypsiferous Soil

Table 7, shows that the (APRD) method has been superior to the (CI) method in the weight of 500 seeds by (0.67)%. These results are consistent with [1,3,21]. As for the levels of addition (γ-PGA), all levels (PG_1, PG_2, PG_3, PG_4) achieved a significant superiority in the weight of 500 seeds compared to the treatment (PG_0). Increasing the level of γ-PGA addition increases soil moisture retention and increases the ability of roots to absorb nutrients, thus increasing productivity [13].

Table 7. Effect of irrigation methods and application level of Poly-γ-glutamic acid (γ-PGA) and the interaction between them on the weight of 500 seeds (gm).

PG/ I	CK	15 kg/h	30 kg/h	60 kg/h	120 kg/h	Average
APRD	169.33	171.67	179.67	190.33	193.00	180.80
CI	167.67	174.67	178.33	185.00	192.33	179.60
Average	168.50	173.17	179.00	187.67	192.67	
LSD	I = 8.2088		PG = 3.1044		I*PG = 4.3902	

As for the effect of the interaction between the factors on the weight of the seeds, the treatments (I1PG2, I1PG3, I_1PG_4, I_2PG_1, I_2PG_2, I_2PG_3, I_2PG_4) achieved a significant increase compared to the treatment (I_2PG_0) with increased rates of 4.19, 6.59, 7.19, 10.77, 13.77, 14.97 and 15.57 %. We note from Table No. (3) that the depth of the irrigation water for the CI method is greater than the depth of the irrigation water for the APRD method, as it was previously explained.

3.6. The Effect of the Effect of Drip Partial Root-Zone Drying and of Poly-γ-Glutamic Acid (γ-PGA) Addition on the Water use Efficiency of Corn Yield in a Gypsiferous Soil

Table 8, shows that the treatment (APRD) has achieved a water use efficiency of (3.25) kg of dry seed yield of yellow corn. m3, with an increase rate of (17.33)% compared to the (CI) treatment, which achieved a use efficiency of (2.77) kg. M3 This result agrees with [21]. The reason for this result could be due to the lower values of water consumption in the (APRD) method and the reduction of water losses through evaporation and loss by deep seepage compared to the (CI) method (Jovanic et al, 2010). Thus, it works to increase the yield and raise the efficiency of use [27,28]. We note that increasing the levels of addition of (γ-PGA) increased the water use efficiency by 8.11, 15.83, 23.17 and 32.43 % compared to the level (PG_0).

Table 8. Effect of irrigation methods and application level of Poly-γ-glutamic acid (γ-PGA) and the interaction between them on the water use efficiency of corn yield.

PG/I	CK	15 kg/h	30 kg/h	60 kg/h	120 kg/h	Average
APRD	2.67	2.94	3.23	3.60	3.94	3.25
CI	2.51	2.67	2.80	2.86	3.03	2.77
Average	2.59	2.80	3.00	3.19	3.43	

The reason for this result could be the superior ability of (γ-PGA) to store and absorb water in amounts thousands of times its own weight [29]. The γ-PGA can be likened to a small water tank And (γ-PGA) can have an effect on the soil's ability to hold water and available water [22]. The overlap treatment (I_1PG_4) achieved the highest value of (3.94) kg. M3 and the lowest value of the transaction (I_2PG_0).

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Effect of Humic Acid on the Phytoremediation of Cd in Contaminated Soil using *Nerium oleander*

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Abstract. The experiment was conducted in Kirkuk-Iraq for the period from 1/3/2022 to 1/9/2022, to study the effect of humic acid in three concentrations (0,5,10) ml. pot^{-1} in the phytoremediation of soil contaminated with cadmium using *Nerium oleander*. the design used in the experiment R.C.B.D, the results showed the efficiency of *Nerium oleander* in removing cadmium from contaminated soil and its accumulation in plant tissues with the support of humic acid. concentration 10 ml. pot^{-1} led to a significant increase in the indicator of cadmium concentration in the shoot and root system (1.21, 0.75) respectively, while in the control plant it reached (0.87, 0.35), respectively. Translocation Factor (TF)>1 for all humic concentrations (1.78, 1.44, 1.45), respectively. concentrate 10 ml. pot^{-1} Humic gave the highest bioaccumulation factor (BAF) value reached 0.76, while it was 0.45 in the control plant. As the concentration exceeds 10 ml. pot^{-1} of humic gave the highest concentration index (CI) value, which ranged between 1.47 and 1.05 in the control plant.

Keywords. Humic acid**,** Phytoremediation, Cadmium, *Nerium oleander.*

1. Introduction

There are two types of sources of heavy metals are natural sources and human-induced (industrial) sources. Natural resources include volcanoes and Parent Material and others [1,2], while the new sources resulting from Various human activities such as mining, industry, agriculture, waste treatment, means of transportation, and others Which results in large amounts of heavy metals to the environment, and in particular releases smelting processes [3,4], Several factors affect the degree of solubility, movement and accumulation of heavy metals reaching the soil, amount in the soil, pH, amount of organic matter and activity of soil microorganisms, soil texture and capacity Cation Exchange and the source of contamination as well as the properties of the element itself [5], In general, the degree of dissolution and solubility of heavy element compounds in the soil increases with a decrease Soil pH values, Where each element has a threshold at which the compounds of this element begin to dissolve, for example the pH threshold Then cadmium compounds begin to dissolve 6.5 [6]. The problem of heavy metal pollution has been exacerbated in recent decades due to its stability and accumulation in the biomedium for a while long period of time [7], As they cannot be biodegraded, they accumulate in the food chain, causing Extremely toxic to plants and animals and a serious threat to human health [8]. Phytoremediation as an environmental concept defined as the use of

plants and microorganisms in the rhizosphere to remove or isolate pollutants, or detoxify them by converting them into less toxic forms [9]. *Nerium oleander* is an evergreen shrub belonging to the Apocynaceae that grows wild in the Mediterranean and southwest Asia, especially in warm regions [10], In general, plants with a high ability to accumulate heavy metals are called Hyperaccumulators, which are plants growing in polluted soils and capable of absorbing heavy metals at high levels and thus accumulating them either in their roots or leaves or branches at very high concentrations compared to other plants [11] Humic acid is an organic acid produced naturally from the humic substance and one of the main components of humus. It consists of a mixture of humate and fulvic. It contains nitrogen, oxygen and hydrogen in varying proportions, which results in compounds with varying molecular weights. It is one of the economical commercial products with fast effectiveness and harmless to humans and animal and plant [12] Humic acid support to increase the readiness of nutrients and improve their availability and absorption by the plant, as humic works as a medium for transferring nutrients from the soil to the plant, which leads to an increase in the strength of the growth of the root system and its lateral branches, and increases the number of beneficial microorganisms in the soil and increases the protein content of the plant [13], In addition, humic chelates nutrients subject to leaching and releases them slowly and for a longer period, improving plant nutrition and soil structure in agricultural terms [14].

2. Materials and Methods

2.1. Sample Collection

The experiment was conducted in the Agricultural Research and Experiment Station - College of Agriculture - University of Kirkuk, for the period from 1/3/2022 to 1/9/2022 To study Effect of Humic Acid on the Phytoremediation of Cd in Contaminated Soil using *Nerium oleander,* Humic in three concentrations (0,5,10) $ml.pot^{-1}$, The seedlings were planted in pots with a diameter of 29 cm, a height of 30 cm. The soil used in the experiment was taken from an agricultural field in Wadi al-Naft - Kirkuk Governorate, In the experiment 270 pots were used with three replications, and each replicate contained 90 pots. The experiment was designed with a Randomized Complete Block Design (R.C.B.D). samples were collected six months after planting, then the plants were cleaned, dried, and the samples were taken for chemical analysis. Data were collected and analyzed using the statistical program (SAS) according to Dunkin's multiple test at a probability level of 5%.

2.2. Indicators of Plant Efficiency in Extracting Heavy Metals

2.2.1. Concentration of cadmium (Cd) in the Plant (Shoot - Root System)

Wet digestion method was used to prepare plant samples (leaves, stems and roots) according to the method reported by Elmer [15], The dried plant samples were grinding and 2 gm were taken, then 10 ml of concentrated (HNO_3) was added to it, then heated on an electric heater and the samples were left to cool, then 2-4 ml of ($HClO_4$) with a concentration of 70% were added to it, the samples were heated again, The samples were left to cool down, and then transferred to a beaker with a capacity of 50 ml, The volume was filled with distilled water, so that the final volume of the plant samples was 50 ml. An Atomic absorption spectrophotometer was used to estimate the concentration of cadmium Cd in plants.

2.2.2. Translocation Factor (TF)

The plant Translocation factor values were calculated by dividing the concentration of heavy elements in the shoot by the concentration of heavy metal in the root system [16], as in the following equation:

$$TF = HM_S / HM_R$$

whereas: HM_S = concentration of heavy metals in shoots; HM_R = concentration of heavy metals in root system.

2.2.3. Bioaccumulation Factor (BAF)

The Bioaccumulation Factor values were calculated by dividing the concentration of heavy metals in the plant by the concentration of heavy metals in the soil [17], as in the following equation:

$$BAF = HM_{plant} / HM_{Soil}$$

whereas: HM_{plant} = concentration of heavy metals in plant; HM_{Soil} = concentration of heavy metals in soil.

2.2.4. Concentration Index (CI)

Its values are calculated by dividing the concentration of heavy metals in the treated plant by the concentration of heavy metals in the control plant [18], as in the following equation:

$$CI = HM_{PLANT} / HM_{CONTROL}$$

whereas: HM_{PLANT} = concentration of heavy metals in plant; $HM_{CONTROL}$= concentration of heavy metals in control plant.

3. Results and Discussion

3.1. Cadmium Concentration in Shoot

The results showed in Figure (1) that there was a significant superiority in the humic treatment in concentration of cadmium in the shoot, as the concentration exceeded 10 ml.pot^{-1} humic and reached 1.21 ppm, while it reached 0.87 ppm in control plant, the reason can be attributed to the fact humic acid contains chelating elements to different ions in the soil by forming carboxylic and phenolic-OH groups which are the dominant humic binding (chelating) groups [19].

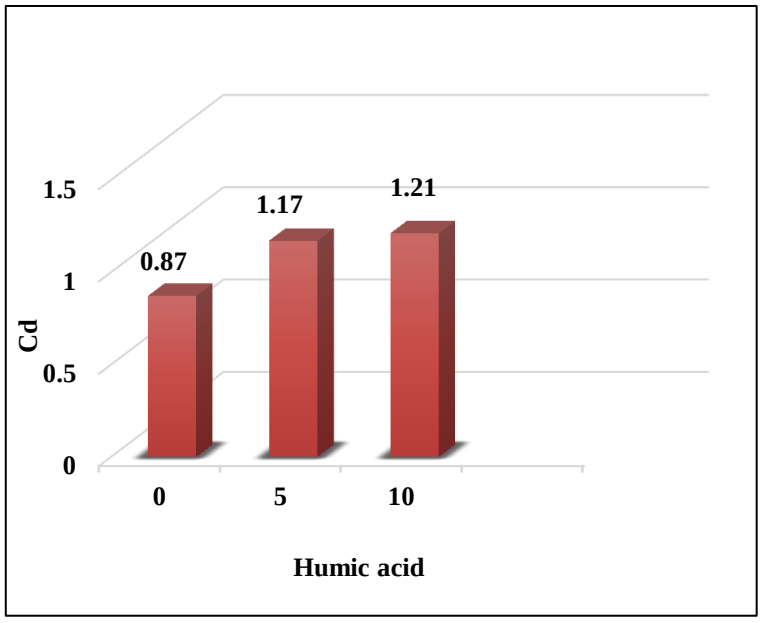

Figure 1. Cadmium concentration in shoot.

3.2. Cadmium Concentration in Root System

The results in Figure (2) showed that there were significant differences for the addition of humic in the concentration of cadmium in the root system, as the concentration of 10 ml.pot^{-1} humic exceeded the rest of the treatments, amounting to 0.75 ppm, and in the control plant it reached 0.53 ppm, The reason may be attributed to the fact that it is the plant with support from some proteins and enzymes produced or secreted by microorganisms in the Rhizosphere, in which the humic contributes to the activity of these organisms, and a symbiotic relationship is established between the roots and the

microorganisms, and the formation of a suitable environment for the growth of roots and the absorption of nutrients and heavy metals by the roots [20].

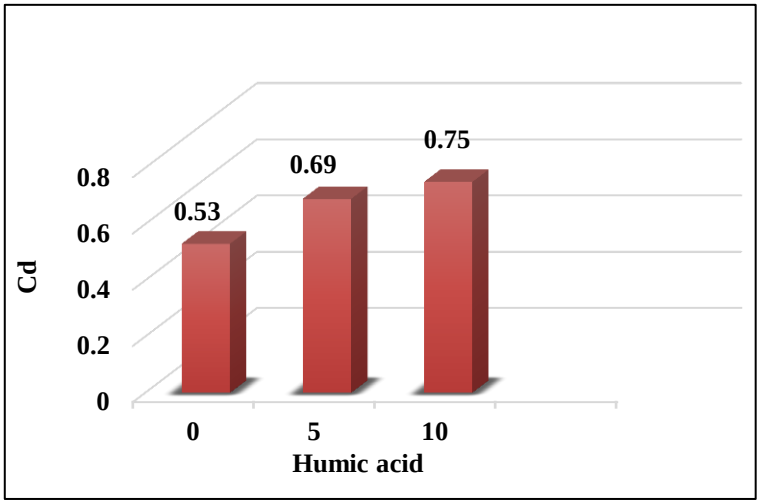

Figure 2. Cadmium concentration in root system.

3.3. Translocation Factor (TF)

The results showed in Figure (3) that all values of the Translocation Factor TF >1 for all humic concentrations with the superiority of the control plant over the rest of the concentrations amounted to 1.78 <1. This result is similar to what was mentioned by Al-Bayati [21] who showed the possibility and ability of some types of plants to grow in Contaminated soils absorb heavy metals through their spreading roots and then transfer them to the upper parts of the plant, this process depends on the type of plant, nature of the element, and the ability of the plant to withstand high concentrations of heavy elements.

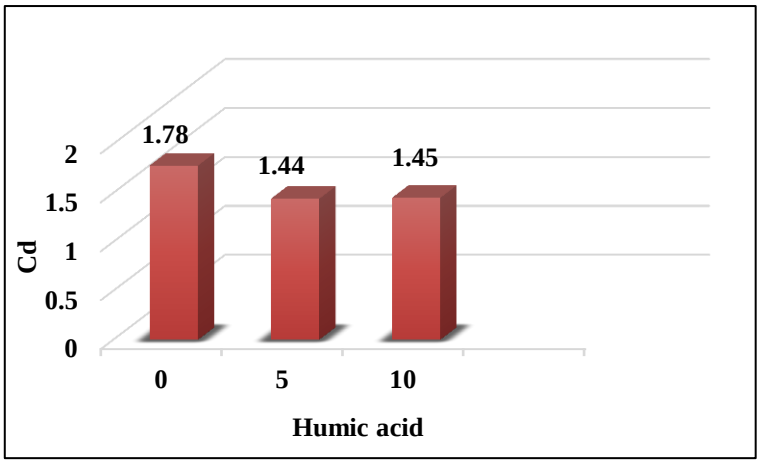

Figure 3. Translocation Factor (TF).

3.4. Bioaccumulation Factor (BAF)

The results show in Figure (4) that there are significant differences in the addition of humic in BAF to cadmium, as the concentration exceeded 10 ml. pot^{-1} humic reached 0.76, while in the control plant it reached 0.45, the reason can be attributed to humic solutions that increase the value of BAF through the removal of heavy metals from contaminated soil due to humic acid functions which effectively bind heavy metals and remove them from the soil system [22].

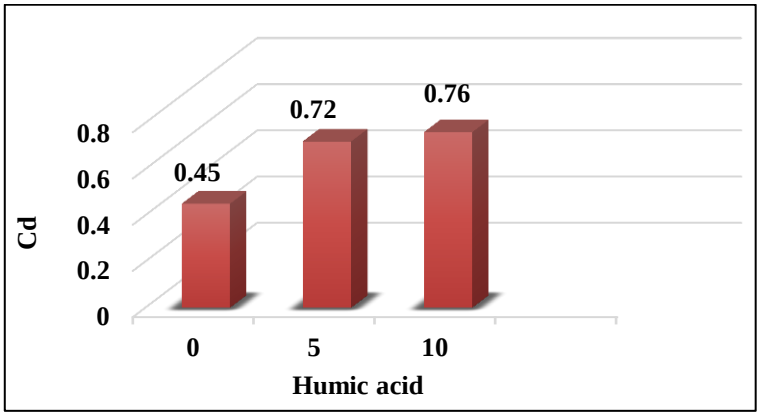

Figure 4. Bioaccumulation Factor (BAF).

3.5. Concentration Index (CI)

The results in Figure (5) show that there are significant differences when adding humic in the Concentration Index (CI)concentration index of the cadmium, as the concentration exceeded 10 ml.pot^{-1} humic reached 1.47, while in the control plant it reached 1.05, the reason can be attributed to the fact that heavy metals, although Not readily bioavailable, can be retained in the soil in the form of aggregates associated with humic molecules, which can be easily displaced by root secretions [23].

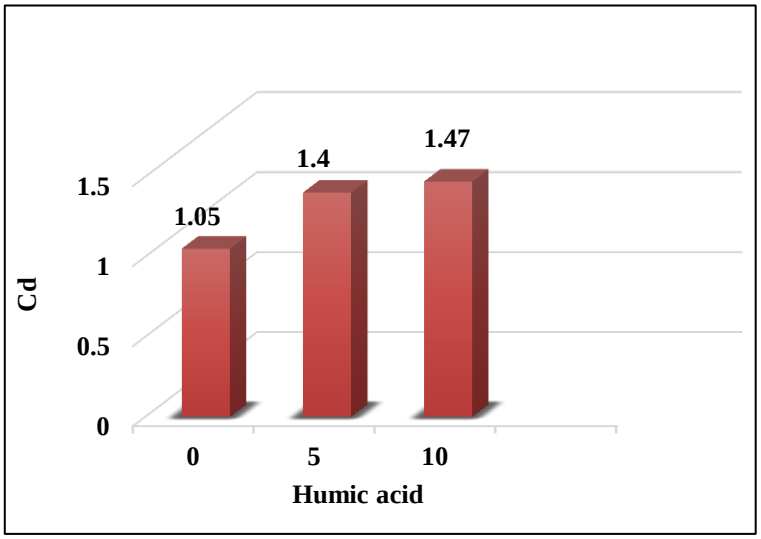

Figure 5. Concentration Index (CI).

Conclusion

The use of humic in plant treatment increased the efficiency of the *Nerium oleander* in removing cadmium from contaminated soil and accumulating it within the plant tissue (roots, stem, leaves), and reducing the concentration of cadmium in the soil with the support of humic, which led to a decrease in the pH and improved soil structure , increased activity of microorganisms and stimulate the transfer of nutrients and heavy metals by chelating group these elements and making them available and facilitating their absorption by the roots of the plant and the success of the process of phytoremediation.

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Effect Foliar Application of Kinetin, Boron and Organic Fertilizer on Yield and Quality of Fig cv. White Adriatic

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Abstract . This research was conducted on a private fig orchard at Kirkuk governorate to study effect of (3) level from organic fertilizer at(0,5,10 ml. L^{-1}) and foliar spraying with kinetin at (0,150,300 mg. L^{-1}) and boron (0, 40, 80 mg. L^{-1}) and interaction on some characteristics and yield. experiment was design adopted according randomized complete block design R.B.C.D. with(3)replicates. Duncan test was used to compared means, at the level 5%. Result showed that treatment of organic 10 ml.L^{-1}+kinetin 300 mg.L^{-1} + Boron 80 mg.L^{-1} caused a best result of all treatment in this experiment.

Keywords. Fig c.v white Adriatic, Organic fertilizer, Kinetin, Boron.

1. Introduction

Carica ficus L. belongs to Moraceae family, name comes from western Anatolia region, which famous for cultivation of figs. It was spread in Mediterranean countries such as Spain, Portugal and southern Franc [1,2]. Fruits are used as fresh and dried fruits, juices, wine. latex substance is also used in the manufacture of cheese[3]. Most of active substances in figs have antiseptic and laxative properties. it helps in digestion, treatment of some intestinal diseases, chronic constipation [4].

Use of organic materials of animal or plant origin as a source of fertilizer is becoming increasingly popular around the world as a means of mitigating chemical pollution of agricultural soils, increasing the availability of chemically-free food for humans and other animals, and making up for the depletion of soil organic matter caused by widespread crop production [5]. Organic matter is an important source of improved soil structure, provides necessary nutrients for plants' growth[6,7]. Consider a cheap source to improve soil's various properties[8].

Boron is an essential micronutrient element, it was proven in 1910[9], it has a role in cell division, formation of sugar complex substances, facilitating movement and transfers to storage sites in fruits, as well regulatory role through regulating plant hormones [10,11].

Cytokinines were discovered in 1950s by Carlos Miller. They have ability to boost plant cell division[12]. Involved in wide range of developmental processes including germination and leaf senescence. cytokinin has a substantial role in the formation of nitrogen-fixing nodules and other plant-microbe interactions [13,14] . [15] found that adding (10 kg.$plant^{-1}$) of organic fertilizer gave a best result in fruit size, weight, length yield on fig. another study[16] concluded that organic manners (10 kg. $Plant^{-1}$) caused an important increase in fig trees at fruit weight, diameter, size, and length.[17] concluded foliar spraying (Boron 2 ml.L^{-1} + Calcium 5 ml.L^{-1}) on fig cv. Sultani, led to a significant

increase in fruit size, weight, length, diameter, yield [18], found sprayed mango with boron at 0.2% gave a best result in fruit weight, length, size, diameter, yield.[19] found spray of Zeatin and Grofalcs at 250mg.L^{-1} gave a best significant increase in fruit weight, size, hardness, yield on fig.[20], found spray of BA at 40 mg.L^{-1} on fig caused excellent result at fruit weight, size, yield.

2. Materials and Methods

The research was carried out in Kirkuk, on an orchard of fig trees that were planted at distance of 5 × 5. Trees were chosen with similar growth as much as possible, agricultural operations were executed and trees were treated 3 times, starting 8, 9,10/5/2022.treatments included the following:

2.1. Organic Fertilizer (Travert Evo)

- F0 - control
- F1 - Add 5 ml L^{-1}
- F2 - Add 10 ml L^{-1}

2.2. Kinetin

- K0- Control
- K1 - Spraying 150 mg L^{-1}
- K2 - Spraying 300 mg L^{-1}

2.3. Boron

- B0 -Control
- B1 - Spraying 40 mg L^{-1}
- B2 - Spraying 80 mg L^{-1}

Table 1. Analysis of physical and chemical of the soil samples.

Properties	Unit	Values
EC	Mmho.cm^{-1}	0.19
pH	———	7.22
Dissolved Solids	mg/kg	100
Nitrogen	mg/kg	630. 45
Phosphorous	mg/kg	25.74
Potassium	mg/kg	161.22
Lime	%	24.125
Organic Matter	%	2.404
Texture	———	clay
Clay	%	28
Silt	%	46
Sand	%	26

Table 2. Composition of organic fertilizer(Taravert Evo).

Declared Content
Total (N) Nitrogen 7% W/W
Organic Nitrogen (N) 3.4% W/W
Water-Soluble Potassium Oxide (K2o) 3% W/W
Seaweed Extracts 6% W/W
Total Amino Acids 17% W/W
Organic Matter 15% W/W
Free L Amino Acids 10% W/W

2.4. Studied Measurements

- Leaf area**:** twenty Leaves are picked per tree from middle shoots, and leaf area measured the outlined by [21] Leaf area (cm^2) = 0.3 (0.79 × diameter^2) + 76.71.
- Fruit weight (g): fruits were weighed by an electric scale.
- Fruit volume (cm^3): volume was estimated by using a cylinder.
- Fruit length (cm): length was measured by using (vernier).
- Fruit diameter (cm): fruit was measured by using (vernier).
- Fruit shape : It was calculated by dividing length/diameter.
- Firmness(kg/cm^2): penetrometer was used to measure the hardness.
- Fruits number: calculated by divided yield on average weights fruit.
- Total yield($kg.tree^{-1}$): yield was calculated by weight of the fruit.

3. Results and Discussion

3.1. Leaf Area

Table (3) showed a high concentration of organic 10 $ml.L^{-1}$, kinetin300 mg. L^{-1} ,Boron 80 $mg.L^{-1}$ which recorded a highly result in leaf area 239.48 cm^2, 238.92 cm^2, 237.76 cm^2 compared to untreated witch gave lowest value 226.87 cm^2, 226.02 cm^2, 228.63 cm^2 separately. As for combination of organic10$ml.L^{-1}$ + kinetin 300 mg. L^{-1}, kinetin300 $mg.L^{-1}$ + Boron 80 $mg.L^{-1}$ and organic10 $ml.L^{-1}$ + Boron80 $mg.L^{-1}$ caused a highly significant increment 249.65cm^2, 243.69 cm^2, 243.05 cm^2. while control recorded lowest value 218.13 cm^2,221.80 cm^2, 221.15 cm^2 respectively. well as, organic10 $ml.L^{-1}$ + kinetin300 $mg.L^{-1}$ + Boron80 mg. L^{-1} led to highest significance which gave154.49 cm^2 while control reached 211.73 cm^2. possibly characteristics increases of vegetative growth is a result of adding organic fertilizer 10 $ml.L^{-1}$ may be due to role increasing permeability of cell membranes and thus increasing absorption of water, nutrients, increasing activation of some enzymes[22]. Which leads to an impact on vital processes such as respiration, photosynthesis [23].

Table 3. Effect of organic fertilizer(Taravert Evo), kinetin, boron and interaction on leaf area for growing season 2022.

Organic fertilizer	**Kinetin(mg. L^{-1})**			**Average organic fertilizer**
	0	**150**	**300**	
0	218.13 g	227.59 c	234.88 f	226.87 c
5	228.08 d	229.87 de	240.23 b	232.72 b
10	231.85 e	239.94 b	249.65 a	240.48 a
Kinetin	Boron (mg. L^{-1})			Average Kinetin
	0	40	80	
0	218.80 g	226.87 f	229.39 e	225.02 c
150	230.78 e	236.41 c	240.21 b	235.80 b
300	235.29 d	237.77 c	243.69 a	238.91 a
Boron	organic fertilizer ($m.L^{-1}$)			Average Boron
	0	5	10	
0	221.15 h	227.96 g	233.48 e	227.53 c
40	229.45 f	234.97 d	236.76 c	233.72 b
80	235.29 d	240.11 b	243.05 a	239.48 a

organic fertilizer	Kinetin								
	0			150			300		
	Boron								
	0	40	80	0	40	80	0	40	80
0	211.73 n	218.84 lm	223.80 kl	233.01 fg	236.56 e	233.20 fg	218.72 lm	222.49 l	241.43 de
5	226.56 jk	231.74 gh	230.94 hi	225.32 jk	230.63 hi	233.67 fg	235.47 ef	242.54 de	243.69 cd
10	227.11 jk	230.02 hi	233.43 ef	234.02 ef	242.03 de	243.77 cd	245.47 c	248.28 b	254.94 a

*Means followed by the same letter(s)within each column during each season are not significantly different at 0.05

3.2. Fruit Weight

Results in Table (4) showed a significant increase weight of fruit as treatment of organic10ml.L^{-1},kinetin 300 mg. L^{-1},boron 80 mg.L^{-1} ,recorded 33.65, 34.19, 35.43 compared to control amounted 30.94, 31.39 35.43, respectively**.** about interaction, it is clear that organic 10ml.L^{-1} + kinetin 300 mg. L^{-1} was significantly superior in value of 38.32 compared to control which gave 30.09. as well as, organic 10ml.L^{-1} + boron 80 mg. L^{-1} recorded a significant increase reached 36.43, while control reached 30.85. also of kinetin 300 mg. L^{-1} + Boron 80 mg. L^{-1} caused different significant increases at 35.21whilst control got lowest value of 30.36. as for treatment of organic10ml.L^{-1} + kinetin300 mg. L^{-1}+ boron80 mg. L^{-1} showed an excellent result by given 38.79, whereas control showed lowest value of 30.00. reason for fruit increases in weight that occurred maybe due to effect of kinetin in increasing leaf area and thus increasing effectiveness of photosynthesis and other enzymatic processes [24] which led to an increase in processed substances in leaves, their transfer to fruits, thus increasing growth and improving characteristics of fruits[25].

Table 4. Effect of organic fertilizer(Taravert Evo), kinetin, boron and interaction on fruit weight for growing season 2022.

Organic fertilizer	**Kinetin(mg. L^{-1})**			**Av. organic fertilizer**
	0	**150**	**300**	
0	30.09 f	30.99 e	31.74 de	30.94 b
5	31.43 e	33.43 c	35.19 b	33.35 a
10	32.44 d	34.88 b	38. 32 a	33.65 a
kinetin	Boron(mg. L^{-1})			Average kinetin
	0	40	80	
0	30.36 f	31.17 ef	32.64 de	31.39 c
150	31.96 e	32.84 de	34.06 bc	32.95 b
300	33.14 cd	34.23 b	35.21 a	34.19 a
Boron	organic fertilizer (m.L^{-1})			Av. Boron
	0	5	10	
0	30.85 e	31.32 de	33.38 cd	31.85
40	31.64 de	32.87 d	35.13 b	33.21
80	34.25 c	35.63 ab	36.43 a	35.43 a

organic fertilizer	kinetin								
	0			150			300		
	Boron								
	0	40	80	0	40	80	0	40	80
0	30.00 j	30.13 ij	31.47 fg	30.34 ij	31.28 fg	31.92 gh	30.84 hi	31.34 fg	34.61 cd
5	30.21 ij	30.65 hi	31.80 gh	32.28 ef	31.53 fg	34.88 cd	32.97 de	36.15 bc	36.30 bc
10	32.57 ef	33.50 de	36.45 bc	32.88 ef	33.84 de	34.46 cd	33.46 de	38.58 ab	38.79 a

*Means followed by the same letter(s)within each column during each season are not significantly different at 0.05

3.3. Fruit Volume

It is clear from table (5) that results showed significant superiority of organic 10 ml .L^{-1}, kinetin300 mg. L^{-1} , Boron80 mg. L^{-1} which gave 32.35, 31.78, 32.18, while control gave the lowest worth 27.45, 27.67, 28.04 respectively. As for interaction, organic 10 ml.L^{-1} + kinetin300 mg.L^{-1} was recorded an excellent value of 35.32 compared to control which gave of 25.21as well as, organic10 ml.L^{-1} + boron80 mg.L^{-1} gave worth 33.96, while control was given 25.72.as for kinetin300 mg.L^{-1} + boron 80

mg.L^{-1} was caused a high significant 32.81 whilst control reached value of 26.14. The reason for an increase in fruit size in trees treated with kitten may be due to the fact that this regulator induces division cells and increases in size[26]. It may be organic fertilizer contains nitrogen that plays an important role increasing vegetative growth by increasing process of photosynthesis, which helped to pull Amounts of nutrients, so size of fruit increased as it is a center of attraction (sink) for products of photosynthesis[27,28]. Boron increases leaf area, thereby increasing the effectiveness of photosynthesis and other enzymatic processes that increase leaf size fruit [29].

Table 5. Effect of organic fertilizer(Taravert Evo), kinetin, boron and interaction on fruit volume for growing season 2022.

Organic fertilizer	**Kinetin(mg. L^{-1})**			**Av. organic fertilizer**
	0	**150**	**300**	
0	25.21 f	26.75 e	30.66 c	27.54 c
5	27.29 de	30.55 c	32.42 bc	30.08 b
10	28.35 d	33.38 b	35.32 a	32.35 a
kinetin	Boron (mg. L^{-1})			Av. kinetin
	0	40	80	
0	26.14 e	27.41 de	29.48 cd	27.67 c
150	28.50 de	29.43 cd	30.14 bc	29.35 b
300	30. 76 bc	31.78 ab	32.81 a	31.78 a
Boron	organic fertilizer (m.L^{-1})			Av. Boron
	0	5	10	
0	25.72 e	28.10 de	30.31 bc	28.04 c
40	25.43 e	29.85 cd	31.97 b	29.08 b
80	29.40 cd	33.20 ab	33.96 a	32.18 a

organic fertilizer	kinetin								
	0			150			300		
	Boron								
	0	40	80	0	40	80	0	40	80
0	23.25 o	24.88 no	27.48 kl	24.32 no	25.55 mn	29.67 hi	25.84 mn	28.59 ij	30.68 gh
5	26.87 lm	27.67 kl	31.30 de	26.12 lm	28.48 ij	30.52 gh	27.83 kl	29.51 hi	32.60 cd
10	28.11 ij	30.84 gh	32.13 cd	29.62 hi	32.58 cd	33.96 bc	31.79 de	35.17 ab	35.80 a

*Means followed by the same letter(s)within each column during each season are not significantly different at 0.05

3.4. Fruit Length

Results in table (6) indicate a significant increase of organic 10 ml.L^{-1}, kinetin 300 mg.L^{-1}, boron 80 mg.L^{-1}, by given 5.33, 4.99, 5.28, compared to control which gave 4.33, 4.29, 4.30 respectively. So we some different significantly between interaction treatment especially organic 10 ml.L^{-1}+kinetin 300 mg.L^{-1} which gave 5.68, also organic 10 ml.L^{-1}+boron 80 mg.L^{-1} reached 5.63, however treated with kinetin 300 mg.L^{-1}+boron 80 mg.L^{-1}5.19 in contrary control treatments. With regarded to organic 10 ml.L^{-1}+ kinetin 300 mg.L^{-1}+boron 80 mg.L^{-1} led to high significant 5.79 while control gave lowest 4.02. reason may be attributed to role of cytokinin, which stimulates nutrients transport, such as amino acids and plant hormones Especially(auxin) [30], as it is properties of cytokines Interference with auxin and thus leads to an increase in size, length, diameter of fruit, a natural effect of auxin is to stimulate elongation cells while natural effect of cytokinin is to stimulate cells division so causes an increase in length, diameter of the fruit[31].

Table 6. Effect of organic fertilizer(Taravert Evo), kinetin, boron and interaction on fruit length for growing season 2022.

Organic fertilizer	Kinetin(mg. L^{-1})			Av. organic fertilizer
	0	**150**	**300**	
0	4.14 f	4.30 ef	4.57 d	4.33 c
5	4.47 de	4.79 cd	5.10 b	4.78 b
10	4.83 c	5.50 a	5.68 a	5.33 a
kinetin	Boron(mg. L^{-1})			Av. kinetin
	0	40	80	
0	4.12 f	4.43 de	4.34 ef	4.29 c
150	4.70 cd	4.51 de	4.62 d	4.61 b
300	4.84 bc	4.95 b	5.19 a	4.99 a
Boron	organic fertilizer (m.L^{-1})			Av. Boron
	0	5	10	
0	4.17 f	4.29 ef	4.45 de	4.30 c
40	4.56 de	4.73 cd	5.21 b	4.83 b
80	4.89 c	5.34 b	5.63 a	5.28 a

organic fertilizer	kinetin								
	0			150			300		
	Boron								
	0	40	80	0	40	80	0	40	80
0	4.02 k	4.16 jk	4.22 ij	4.09 k	4.28 ij	4.19 jk	4.32 hi	4.46 gh	4.65 ef
5	4.33 hi	4.65 ef	4.71 de	4.52 hi	4.41 gh	4.88 de	4.63 ef	4.77 de	5.42 b
10	4.42 gh	4.93 de	4.35 hi	5.03 d	5.11 cd	5.17 bc	5.23 ab	5.61 a	5.79 a

*Means followed by the same letter(s)within each column during each season are not significantly different at 0.05

3.5. Fruit Diameter

It's clear from table(7) significant superiority of organic 10 ml.L^{-1}, kinetin 300 ml.L^{-1}, Boron 80 ml.L^{-1} with values 4.59, 4.28, 4.69 while control recorded lowest values 3.42, 3.47, 3.39 separately. as for interaction treatment of organic 10 ml.L^{-1}+ kinetin 300 ml.L^{-1}, organic 10 ml.L^{-1}+ Boron 80 ml.L^{-1}, kinetin 300 ml.L^{-1}+Boron 80 ml.L^{-1} caused best result with control at 5.32, 5.00, 4.52, individually. reason may be attributed to role of cytokinin, which stimulates nutrients transport, such as amino acids and plant hormones Especially(auxin) as it is properties of cytokines Interference with auxin and thus leads to an increase in size, length, diameter of fruit, a natural effect of auxin is to stimulate elongation cells while natural effect of cytokinin is to stimulate cells division so causes an increase in length, diameter of the fruit [31].

3.6. Fruit Shape

Conclude from table (8) superiority of organic 10 ml.L^{-1}, kinetin 300 mg.L^{-1}, boron 80 ml.L^{-1} which gave values 0.885, 0.866, 0.872 compared to control treatment. While interaction treatment organic 10 ml.L^{-1}+ kinetin 300 mg.L^{-1}, organic 10 ml.L^{-1}+boron 80 ml L^{-1} and kinetin 300 mg.L^{-1}+ boron 80 ml.L^{-1} caused best result and reached 0.933, 0.882, 0.880 respectively. As for organic 10 ml.L^{-1}+ kinetin 300 mg.L^{-1}+ boron 80 ml.L^{-1} showed an important significant amount 0.944 whereas control showed 0.715. reason may be attributed to role of cytokinin, which stimulates nutrients transport, such as amino acids and plant hormones Especially(auxin) as it is properties of cytokines Interference with auxin and thus leads to an increase in size, length, diameter of fruit, a natural effect of auxin is to stimulate elongation cells while natural effect of cytokinin is to stimulate cells division so causes an increase in length, diameter of the fruit[31].

Table 7. Effect of organic fertilizer (Taravert Evo),kinetin, boron and interaction on fruit diameter for growing season 2022.

Organic fertilizer	Kinetin(mg. L^{-1})			Av. organic fertilizer
	0	**150**	**300**	
0	3.19 ef	3.32 ef	3.75 d	3.42 c
5	3.63 de	3.89 cd	4.20 c	3.90 b
10	3.90 cd	4.57 b	5.32 a	4.59 a
kinetin	Boron(mg. L^{-1})			Av. kinetin
	0	40	80	
0	3.15 e	3.48 de	3.79 cd	3.47 c
150	3.76 d	3.73 bc	4.39 b	3.96 b
300	3.95 bc	4.39 a	4.52 a	4.28 a
Boron	organic fertilizer (m.L^{-1})			Av. Boron
	0	5	10	
0	3.28 f	3.41 ef	3.49 ef	3.39 c
40	3.62 de	3.90 d	4.13 cd	3.88 b
80	4.36 c	4.73 b	5.00 a	4.69 a

organic fertilizer	kinetin								
	0			150			300		
	Boron								
	0	40	80	0	40	80	0	40	80
0	3.07 j	3.12 ij	3.18 i	3.25 hi	3.29 hi	3.39 fg	3.44 fg	3.86 ef	3.94 de
5	3.31 fg	3.14 gh	3.79 ef	3. 38 fg	3.33 gh	3.85 ef	3.58 fg	4.02 de	4.10 cd
10	3.74 ef	3.89 de	4.12 cd	4.32 c	4. 05 de	4.78 bc	4.91 bc	5.30 a	5.50 a

*Means followed by the same letter(s)within each column during each season are not significantly different at 0.05

Table 8. Effect of organic fertilizer (Taravert Evo), kinetin, boron and interaction on fruit shape for growing season 2022.

Organic fertilizer	Kinetin(mg. L^{-1})			Av. organic fertilizer
	0	**150**	**300**	
0	0.804 e	0.809 e	0.811 de	0.808 b
5	0.819 cd	0.822 bc	0.831 bc	0.824 b
10	0.852 b	0.871 ab	0.933 a	0.885 a
kinetin	Boron(mg. L^{-1})			Av. kinetin
	0	40	80	
0	0.773 e	0.774 e	0.823 bc	0.790 c
150	0.801 cd	0.824 bc	0.831 ab	0.818 b
300	0.848 ab	0.872 a	0.880 a	0.866 a
Boron	organic fertilizer (m.L^{-1})			Av. Boron
	0	5	10	
0	0.765 d	0.787 c	0.808 cd	0.787 c
40	0.801 cd	0.820 bc	0.815 bc	0.812 b
80	0.851 ab	0.883 a	0.882 a	0.872 a

organic fertilizer	kinetin		
	0	150	300
	Boron		

	Kinetin(mg. L^{-1})								Av. organic fertilizer
	0			150			300		
	0	40	80	0	40	80	0	40	80
0	0.715 h	0.740 gh	0.765 fg	0.731 gh	0.800 ef	0.818 ef	0.718 h	0.864 cd	0.846 de
5	0.767 ef	0.823 de	0.798 ef	0.851 cd	0.786 ef	0.755 hi	0.736 ij	0.842 de	0.818 cd
10	0.814 cd	0.873 de	0.802 de	0.816 cd	0.843 de	0.895 bc	0.924 bc	0.931 ab	0.944 a

*Means followed by the same letter(s)within each column during each season are not significantly different at 0.05

3.7. Firmness

Table(9) showed that organic 10 ml.L^{-1} ,kinetin 300 mg.L^{-1} and Boron 80 mg.L^{-1} with concentrations 0.365, 0.348, 0.358, caused a significant effect while control gave lowest values 0.315, 0.327, 0.321 individually. As for interaction organic10 ml.L^{-1} +kinetin 300 mg.L^{-1} ,organic 10 ml.L^{-1} +Boron 80 mg.L^{-1} ,kinetin 300 mg .L^{-1} + Boron 80 mg.L^{-1} led to a best result by given 0.380, 0.353, 0.369 compared to control. regarding of organic10 ml.L^{-1} + kinetin 300 mg.L^{-1}+ Boron 80 mg.L^{-1} significance by given 0.387 whereas control 0.302.Increasing content of carbohydrates in fruits and their role in increasing durability of cell walls due to the increase in total chlorophyll content and increase in hardness of fruits[32].

Table 9. Effect of organic fertilizer (Taravert Evo), kinetin, boron and interaction on fruit firmness for growing season 2022.

Organic fertilizer	Kinetin(mg. L^{-1})			Av. organic fertilizer
	0	150	300	
0	0.308 i	0.317 h	0.322 g	0.315 c
5	0.332 f	0.338 e	0.343d	0.337 b
10	0.354 c	0.363 b	0.380 a	0.365 a
kinetin	Boron(mg. L^{-1})			Av. kinetin
	0	40	80	
0	0.323 e	0.329 ed	0.331 d	0.327 c
150	0.335 cd	0.341 bc	0.338 c	0.338 b
300	0.343 b	0.350 a	0.353 a	0.348 a
Boron	organic fertilizer (m.L^{-1})			Av. Boron
	0	5	10	
0	0.314 f	0.321 ef	0.328 e	0.321 c
40	0.326 de	0.346 c	0.362 b	0.344 b
80	0.341 c	0.365 b	0.369 a	0.358 a

organic fertilizer	kinetin								
	0			150			300		
	Boron								
	0	40	80	0	40	80	0	40	80
0	0.302 p	0.305 no	0.309 no	0.313 mn	0.316 mn	0.329 kl	0.314 mn	0.326 kl	0.340 fg
5	0.321 lm	0.331 jk	0.334 ij	0.338 hi	0.336 hi	0.341 fg	0.324 kl	0.362 cd	0.380 ab
10	0.342 gh	0.352 ef	0.356 de	0.347 fg	0.354 ef	0.360 de	0.366 cd	0.374 b	0.387 a

*Means followed by the same letter(s)within each column during each season are not significantly different at 0.05.

3.8. Fruits Number

Table (10) data showed significantly different both the organic10 ml.L^{-1}, kinetin 300 mg.L^{-1} and Boron 80 mg.L^{-1} achieved a value of 516.41, 479.67, 517.08 compared to control. as for interaction significant differences can be distinguished, where treatment of organic 10 ml.L^{-1} + kinetin 300 mg. L^{-1}, organic 10 ml.L^{-1} + Boron 80 ml.L^{-1} and kinetin 300 mg. L^{-1}+ Boron 80 ml.L^{-1} were recorded 535.72, 534.21, 492.29 respectively. While control gave a lowest value. Also treatment of organic 10 ml.L^{-1} + kinetin 300 mg. L^{-1}+ Boron 80 ml.L^{-1} gave a high value of 556.00 while control gave 386.54. It increases number of fruits by decreasing physiological activities related to building hydrolytic enzymes such as cellulose that degrades cell wall, thus preventing the formation of the separation layer[33].

Table 10. Effect of organic fertilizer (Taravert Evo), kinetin, boron and interaction on fruits number for growing season 2022.

Organic fertilizer	**Kinetin(mg. L^{-1})**			**Av. organic fertilizer**
	0	**150**	**300**	
0	402.59 f	431.21 e	479.08 d	437.62 c
5	471.72 d	439.87 e	507.39 bc	472.99 b
10	489.24 cd	524.29 ab	535.72 a	516.41 a
kinetin	Boron(mg. L^{-1})			Av. kinetin
	0	40	80	
0	440.29 ef	461.16 bc	424.26 f	441.90 c
150	448.08 cd	452.58 de	482.92 ab	461.19 b
300	467.13 bc	479.59 ab	492.29 a	479.67 a
Boron	organic fertilizer(m.L^{-1})			Av. Boron
	0	5	10	
0	405.26 f	422.43 ef	455.06 d	427.58 c
40	435.97 ef	472.50 cd	508.63 bc	472.36 b
80	492.49 bc	524.56 ab	534.21 a	517.08 a

organic fertilizer	kinetin								
	0			150			300		
	Boron								
	0	40	80	0	40	80	0	40	80
0	386.54 n	415.34 mn	419.91 mn	423.16 lm	426.39 lm	439.06 hi	441.09 jk	454.20 hi	465.89 fg
5	451.44 hi	463.58 gh	481.68 ef	440.40 jk	514.24 ef	482.62 fg	440.36 jk	492.16 de	536.86 ab
10	456.33 hi	474.89 fg	512.58 cd	485.70 ef	491.70 de	522.96 bc	509.73 cd	547.33 ab	556.00 a

*Means followed by the same letter (s) within each column during each season are not significantly different at 0.05.

3.9. Total Yield

Results in table (11) indicate a significant increase in each of organic10 ml.L^{-1}, kinetin 300 mg.L^{-1}, boron 80mg.L^{-1} which gave 18.86, 16.86, 18.68 compared to control. as for Interaction, organic 10 ml.L^{-1} + kinetin 300 mg.L^{-1},organic 10 ml.L^{-1}+boron 80mg.L^{-1}, kinetin 300 mg.L^{-1} + boron 80mg.L^{-1} gave an excellent values of 20.37,17.50, 19,54 whereas control gave less values. For the organic 10 ml.L^{-1} + kinetin 300 mg.L^{-1}+ boron 80mg.L^{-1} reached value of 20.78 whilst control reached 11.61. It increases yield by decreasing the physiological activities related to building hydrolytic enzymes such as cellulose that degrades cell wall, thus preventing the formation of the separation layer [33].

Table 11. Effect of organic fertilizer (Taravert Evo), kinetin, boron and interaction on total yield for growing season 2022.

Organic fertilizer	Kinetin(mg. L^{-1})			Av. organic fertilizer
	0	**150**	**300**	
0	13.18 g	14.03 ef	14.78 e	13.99 c
5	15.47 d	15.75 d	17.85 c	16.35 b
10	17.39 cd	18.82 b	20.37 a	18.86 a
kinetin	Boron(mg. L^{-1})			Av. kinetin
	0	40	80	
0	14.27 e	15.11 de	15.63 d	15.00 c
150	14.95 d	16.53 b	16.55 b	16.01b
300	16.17 bc	16.93 ab	17.50 a	16.86 a
Boron	organic fertilizer(m.L^{-1})			Av. Boron
	0	5	10	
0	13.51 f	14.47 e	14.70 e	14.22 c
40	15.95 d	16.74 bc	17.44 b	16.71 b
80	17.24 b	19.27 a	19.54 a	18.68 a

organic fertilizer	kinetin								
	0			150			300		
	Boron								
	0	40	80	0	40	80	0	40	80
0	11.61 k	12.20 jk	12.72 j	12.64 jk	13.95 hi	13.82 hi	13.28 ij	14.25 hi	14.58 h
5	14.62 h	14.53 h	15.76 g	14.27 hi	16.57 fg	16.41 fg	15.95 g	16.60 fg	17.14 ef
10	16.00 fg	8.56 cd	18.40 cd	17.95 de	19.09 c	19.44 bc	20.16 ab	20.19 ab	20.78 a

*Means followed by the same letter (s) within each column during each season are not significantly different at 0.05.

Conclusion

Result indicated that treatment organic 10 ml.L^{-1}+kinetin 300 mg.L^{-1} + Boron 80 mg.L^{-1} showed a highly significant of all treatment in this study. That Showed due role organic to provide studied characters beside plant growth regulators and micronutrients and we advise to use it.

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Effect of Genotype and Fertilization with Nano-Boron on the Growth and Yield of *colored pepper* under Unheated Greenhouse Conditions

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Abstract. The experiment was carried out during the fall season 2021-2022 at the research station of the Department of Horticulture and Landscape Engineering at the College of Agriculture / University of Diyala. To study the effect of genotype and fertilization with boron on the growth and yield of colored pepper under unheated greenhouse conditions. The study included two factors: the first factor was the genotypes, and it included four structures, namely (Carisma Improved green pepper F1, Golden California Wonder pepper V1, Big Red pepper V2, and Orange Sun pepper V3). The second factor is foliar fertilization with boron, and the comparison treatment included (spraying with distilled water only), spraying with normal boron at a concentration of 50 mg L^{-1} and nano-boron at a concentration of 1 mg L^{-1} and nano boron at a concentration of 2 mg L^{-1}, the split board system (S.P) system was applied in the randomized complete block design (RCBD). Traits were studied: plant height, number of leaves, chlorophyll concentration in leaves, number of fruits per plant, fruit size, and total yield of the plastic house.The results of the experiment showed that orange sun had a significant superiority in chlorophyll in leaves (288.8 mg per 100 gm-1 fresh weight), and plant height (56.98 cm). fruit-1), while the two cultivars big red and orange sun excelled in fruit size (89.87 and 89.20 cm 3) respectively, while the golden California wonder cultivar had the highest greenhouse yield (8.607 tons.greenhouse^{-1}). The results of the experiment also indicated that there was a significant effect when fertilizing with boron, as the level exceeded 1 mg L^{-1} nano-boron in chlorophyll in leaves (272.3 mg per 100 gm^{-1} fresh weight), plant height (56.21 cm), and number of leaves (207.6 leaf $^{-1}$) The number of fruits (33.38 fruits.plant^{-1}), and the total yield of the plastic house (8.963 tons. Plastic house^{-1}).

Keywords. Coloured pepper, Nano and normal boron, Vegetative growth, Yield.

1. Introduction

Pepper (*Capsicum annum* L.) is one of the main crops of the Solanaceae family (Solanaceae) and comes in fourth place after tomato, cucumber and eggplant as a crop grown in a protected environment [1]. Colored peppers are among the most important vegetables in the world because they contain flavonoids and phenolic acids, in addition to beta-carotene, anthocyanins, and lycopene, which are beneficial compounds of human health importance. The peppers are eaten cooked or fresh, and the addition of yellow and red peppers improves the quality of the salad and its culinary properties [2]. There are several ways to raise and improve agricultural crops, and the method of import

(introduction) is one of the easiest, especially in countries that do not have sufficient experience and scientific and material capabilities in this field. Therefore, testing the performance of genotypes (varieties, hybrids, or strains) before adopting them for cultivation is necessary due to the large number of production of these genotypes by seed-producing companies and the wide range of genetic variations that characterize them [3].

Nano fertilizers significantly improve plant growth performance and enhance crop production and obtain high quality fruits, the use of non-nano-nutrients globally is mostly dependent on industrial chemical fertilizers which are usually not friendly to the environment and humans and are expensive for farmers [4]. Nanotechnology improves plant uptake of nutrients by regulating the availability of fertilizers and the speed at which they are absorbed by the leaves. It increases stress resistance by improving its uptake capacity and increasing plant defense mechanisms against stress [5; 6].

Boron is one of the most essential mineral nutrients for plants because of its role in controlling the degree of water absorption and the movement of sugars within the plant to its storage locations. In addition to its role in regulating the concentration of nutrients, including nitrogen, phosphorus, potassium and calcium, in the plant in order to obtain an ideal growth for them[7] In a study to find out the effect of genotypes on the yield of sweet pepper included four genotypes of sweet pepper. The results showed that the Denver genotype was distinguished by the number of fruits, the average weight of the fruit, the plant yield, and the total sun yield. In an experiment conducted by [8] ,and in a study [9] to evaluate the growth, production, and quality of fruits of three sweet red pepper varieties (Barbero, Ferrari, and Imperio), the results of the study showed that the variety "Ferrari" excelled in vegetative growth characteristics over the cultivars "Barbero" and "Imperio"[10]. indicated in a study evaluating four genotypes of chili pepper plants that showed that there were significant differences for most of the studied traits, as the Laungi-hybrid variety recorded the highest number of fruits (0.99 fruit.plant^{-1}) and the weight of one fruit was 0.84 gm^{-1} [11]. indicated in a study that he conducted that spraying sweet pepper plants with boron at three levels (0, 10, and 20) mg L^{-1} led to a significant increase in the characteristics (plant height, leaf number, leaf chlorophyll content).[12] conducted an experiment to study the effect of foliar application of boron (B) on the growth and yield of green pepper MIPC^{-1} variety. Five concentrations of boron were used (0, 50, 100, 150 mg.L^{-1}). The results of the study showed that the best fruit weight was 278 gm and the number of fruits (19.0 fruit.plant^{-1}), when boron was added at a concentration of 150 mg.L^{-1}.

The aim of this study was to find out the best genotype of pepper and the best concentration of nano-boron that must be added to the plant in order to obtain the best vegetative growth and yield in quantity and quality.

2. Materials and Methods

The experiment was carried out during the fall season 2021-2022 at the research station of the Department of Horticulture and Landscape Engineering at the College of Agriculture / University of Diyala, to study the effect of genotype and fertilization with regular and nano boron on the growth and yield of colored peppers under unheated greenhouse conditions. The soil of the plastic house was prepared for cultivation in terms of tillage, smoothing, levelling, and the addition of recommended fertilizer to the soil.

8 grooves were made along the plastic house, at a distance of 0.8 m. T-Tape irrigation pipes were placed inside, and the soil of the house was covered with black nylon to prevent the growth of bushes and reduce the loss of irrigation water. Plants were planted on one side of the irrigation pipe, with a distance of 0.4 m between one plant and another.

The seeds of the genotypes of pepper were sown on 8/25/2021 in cork dishes with a capacity of 209 units in a private nursery, and after the seedlings reached the stage of 4-5 true leaves, they were transferred to the plastic house on 10/5/2021 and all service operations were performed from Irrigation and control of weeds and insects equally for all units of the experiment. Study factors: The study included two factors:

First: the genotypes, which included four genotypes, which are (Carisma Improved green pepper produced by the Spanish company Fito, the Golden California Wonder pepper produced by the

American company AMAKA, the Big Red pepper produced by the American company AMAKA, and the Orange Sun pepper produced by the American company AMAKA.
Second: Foliar fertilization with boron element, which included the following: Comparison treatment: spraying with distilled water only and spraying with normal boron at a concentration of 50 mg.L^{-1} produced by Avonchem UK in the form (BH3O3) and spraying with nanoparticles at a concentration of 1 mg L^{-1} and spraying with nano-boron at a concentration of 2 mg.L^{-1} in the form of(BH3 O3) nanoparticles.

2.1. Studied Characteristics
1. Leaves Content of Total Chlorophyll (mg per 100 g.fresh weight^{-1}).

Total chlorophyll was estimated according to the method of [13].

2.2. Plant Height (cm)
The height of the plant was measured using a tape measure, starting from the site of plant contact with the soil, to the highest growing apex of the plant for five plants, and then the average was calculated.

2.3. The Number of Leaves (leaf.plant^{-1})
The number of leaves was calculated for the five plants and for each experimental unit for five plants, then the average was calculated.

2.4. Number of Fruits (fruit^{-1})
The number of fruits per experimental unit for whole yield was calculated in a cumulative manner and the average was calculated according to the following equation:

Number of fruits plant^{-1} = number of fruits of the experimental unit / number of plants in the experimental unit

2.5. Fruit Size (cm^3)
This characteristic was measured by the water displacement of ten fruits taken randomly from each experimental unit, then the average was extracted for one fruit.

2.6. Total Yield (ton.plastic house^{-1})
The total yield of the greenhouse was calculated by multiplying the yield of one plant by the number of plants in the greenhouse (1120 plants). Note that the dimensions of the plastic house are 56 x 9 m and its area is 504 m^{-2}.
Data were taken and results were analyzed using SAS software, and averages were compared using Duncan's multiple range test at a probability level of 0.05.

3. Results

3.1. Chlorophyll Content in Leaves (mg 100gm.fresh weight^{-1})
The results of Table 1 showed that there was a significant effect of the cultivar on the chlorophyll content of the leaves, as the orange sun (V4) plants were characterized by the highest content of 288.8 mg 100 gm^{-1} fresh weight, while the plants of the Big Red variety V3 recorded the lowest value amounting to 217.8 mg 100 gm^{-1} fresh weight.
The results of the table above indicate that there are significant effects of boron fertilization treatments on the trait, as the plants treated with nanoparticle boron concentration of 1 mg L^{-1} (F2) were characterized by the best content of chlorophyll in the leaves amounting to 272.3 mg^{-1} 100 g^{-1} fresh weight, while this value decreased to 220.4 100 mg^{-1} fresh weight in non-fertilized plants (F0).
The results showed in the same table that there were significant effects of the interaction between the cultivar and the foliar fertilization with boron in the trait, as the treatment of Orange sun plants with nanoparticles of boron concentration of 1 mg L^{-1} (V4F2) excelled with the highest content of chlorophyll in the leaves reaching 355.3 mg 100 gm^{-1} fresh weight. While the non-fertilized Big Red

cultivar (V3F0) plants recorded the lowest value of chlorophyll in the leaves, amounting to 5214 mg 100 gm^{-1} fresh weight.

Table 1. The effect of cultivar and boron fertilization and their interactions on the leaf chlorophyll content (mg 100 gm^{-1} fresh weight) for four genotypes of colored pepper*.

Boron fertilization Varieties	**Comparison without composting (F_0)**	**Metallic boron 50 mg L^{-1} (F_1)**	**Nano boron 1 mg L^{-1} (F_2)**	**Nano boron 2 mg L^{-1} (F_3)**	**Varieties average**
Carisma Improved (V_1)	233.4 fg	248.2 e	273.5 c	262.4 d	254.4 B
Golden California Wonder (V_2)	217.7 i	224.8 h	237.6 f	232.2 g	228.1 C
Big Red (V_3)	214.5 i	216.0 i	222.6 h	218.0 i	217.8 D
Orange Sun (V_4)	216.1 i	263.9 d	355.3 a	320.1 b	288.8 A
Boron fertilization averages	220.4 D	238.2 C	272.3 A	258.2 B	

* Means that take the same letter for each factor or for the interaction between them are not significantly different ($P \leq 0.05$) according to Dunkin's multiple range test.

3.2. Plant Height (cm)

The results of Table 2 showed that there was a significant effect of the cultivar on plant height. The plants of cultivar V3 were characterized by the highest height (57.68 cm), while the plants of cultivar V2 recorded the lowest value (45.92 cm).

The results of the above table indicate that there are significant effects of boron fertilization treatments on the trait, as the plants treated with boron (F2) were characterized by the best height reaching (56.27 cm), while this value decreased to (50.83 cm) in non-fertilized plants (F0).

The interaction between cultivar and foliar fertilization with boron significantly affected the trait. The plants of cultivar (V3) were treated with nano-boron (V3F2) with the highest height of (60.44 cm), while the plants of the non-fertilized variety (V2F0) recorded the lowest value of (44.03 cm).

Table 2. Effect of cultivar and boron fertilization and their interaction on plant height (cm) for four genotypes of colored pepper*.

Boron fertilization Varieties	**Comparison without composting (F_0)**	**Metallic boron 50 mg L^{-1} (F_1)**	**Nano boron 1 mg L^{-1} (F_2)**	**Nano boron 2 mg L^{-1} (F_3)**	**Varieties average**
Carisma Improved (V_1)	49.88 g	53.12 f	56.48 cd	55.15 de	53.66 B
Golden California Wonder (V_2)	44.03 j	45.29 ij	47.80 h	46.57 hi	45.92 C
Big Red (V_3)	55.36 de	56.10 cd	60.44 a	58.84 ab	57.68 A
Orange Sun (V_4)	54.03 ef	56.18 cd	60.36 a	57.33 bc	56.98 A
Boron fertilization averages	50.83 D	52.67 C	56.27 A	54.47 B	

* Means that take the same letter for each factor or for the interaction between them are not significantly different ($P \leq 0.05$) according to Dunkin's multiple range test.

3.3. Number of Leaves (leaf^{1})

The results of Table 3 showed that there was a significant effect of the cultivar on the number of leaves, as the plants of cultivar (V1) were characterized by the highest number of leaves amounting to

214.3 leaf.plant^{-1}, while the plants of cultivar (V3) recorded the lowest value, amounting to 140.5 leaf.plant^{-1}.

The results of the above table indicate the presence of significant effects of boron fertilization treatments on the trait, as the plants treated with nanoparticle boron (F2) were characterized by the best value of 207.6 leaf.plant^{-1}, while this value decreased to 169.6 leaf.plant^{-1} in non-fertilized plants (F0).

The results illustrated in the same table that there were significant effects of the interaction between the cultivar and the foliar fertilization with boron on the trait, the plants of the cultivar (V2 F2) excelled with the highest number of leaves reaching 231.1 leaf.plant^{-1}, while the plants of cultivar V3 (non-fertilized (V3F0) recorded the lowest value of 127.6 leaf.plant^{-1}.

Table 3. The effect of cultivar and boron fertilization and their interaction on the number of leaves for four genotypes of colored pepper*.

Boron fertilization Varieties	**Comparison without composting (F_0)**	**Metallic boron 50 mg L^{-1} (F_1)**	**Nano boron 1 mg L^{-1} (F_2)**	**Nano boron 2 mg L^{-1} (F_3)**	**Varieties average**
Carisma Improved (V_1)	199.7 d	209.7 c	225.8 b	221.8 b	214.3 A
Golden California Wonder (V_2)	182.8 f	196.3 de	231.1 a	205.3 c	203.9 B
Big Red (V_3)	127.6 j	140.3 i	152.2 h	141.8 i	140.5 D
Orange Sun (V_4)	168.2 g	192.5 e	221.2 b	196.1 de	194.5 C
Boron fertilization averages	169.6 D	184.7 C	207.6 A	191.3 B	

* Means that take the same letter for each factor or for the interaction between them are not significantly different ($P \leq 0.05$) according to Dunkin's multiple range test.

3.4. Number of Fruits (fruit.plant^{-1})

The results of Table 4 showed a significant effect of the cultivar on the number of fruits, as the plants of cultivar V1 were characterized by the highest number of 33.27 fruits.plant^{-1}, while the plants of cultivar V2 recorded the lowest number of 17.82 fruits.plant^{-1}.

The results of the above table indicate that there are significant effects of boron fertilization treatments on the trait, as the plants treated with nanoparticle boron (F2) were characterized by the best number of fruits amounting to 33.38 fruits.plant^{-1}, while this value decreased to 16.63 fruits.plant^{-1} in non-fertilized plants (F0).

The interaction between the cultivar and foliar fertilization with boron had a significant effect on the trait, as the treatment of cultivar (V1) with nanoparticle boron (V1F2) excelled with the highest number of fruits reaching 44.47 fruits.plant^{-1}, while the non-fertilized (V2) plants were recorded (V2F0) The lowest value was 13.78 for the fruit.plant^{-1}.

3.5. Fruit Size (cm^3)

The results of Table 5 showed that there was a significant effect of the cultivar on the fruit size, as the plants of cultivar V3 had the largest fruit size of 89.87 cm^3, while the plants of cultivar V1 had the lowest fruit size of 64.16 cm^3.

The results of the above table indicate that there are significant effects of boron fertilization treatments on the trait, as the plants treated with regular boron (F1) were characterized by the best fruit size of 84.47 cm^3, while this value decreased to 73.98 cm^3 in non-fertilized plants (F0).

The results of the same table indicate that there were significant effects of the interaction between the cultivar and foliar fertilization with boron in the trait, as the treatment of cultivar (V2) with nanoparticle boron (V2F2) excelled with the largest fruit size of 92.30 cm^3, while the non-fertilized (V4) plants recorded (V4F0) The lowest value was 64.43 cm^3.

Table 4. The effect of cultivar and boron fertilization and their interaction on the number of fruits for four genotypes of colored pepper*.

Boron fertilization Varieties	Comparison without composting (F_0)	Metallic boron 50 mg L^{-1} (F_1)	Nano boron 1 mg L^{-1} (F_2)	Nano boron 2 mg L^{-1} (F_3)	Varieties average
Carisma Improved (V_1)	19.27 ef	31.67 bc	44.47 a	37.69 ab	33.27 A
Golden California Wonder (V_2)	13.78 f	17.22 ef	20.58 ef	19.69 ef	17.82 C
Big Red (V_3)	16.30 ef	24.19 cde	32.69 bc	30.15 bcd	25.83 B
Orange Sun (V_4)	17.18 ef	22.25 def	35.79 b	31.16 cd	26.59 B
Boron fertilization averages	16.63 C	23.83 B	33.38 A	29.67 A	

* Means that take the same letter for each factor or for the interaction between them are not significantly different ($P \leq 0.05$) according to Dunkin's multiple range test.

Table 5. Effect of cultivar and boron fertilization and their interaction on fruit size (cm^3) for four genotypes of colored pepper*.

Boron fertilization Varieties	Comparison without composting (F_0)	Metallic boron 50 mg L^{-1} (F_1)	Nano boron 1 mg L^{-1} (F_2)	Nano boron 2 mg L^{-1} (F_3)	Varieties average
Carisma Improved (V_1)	59.95 j	65.86 hi	85.81 de	84.30 e	64.16 C
Golden California Wonder (V_2)	67.53 h	86.22 d	92.30 a	91.86 ab	75.79 B
Big Red (V_3)	64.74 i	81.08 f	91.10 abc	90.82 abc	89.87 A
Orange Sun (V_4)	64.43 i	70.03 g	90.26 bc	89.84 c	89.20 A
Boron fertilization averages	73.98 D	84.47 A	81.94 B	78.64 C	

* Means that take the same letter for each factor or for the interaction between them are not significantly different ($P \leq 0.05$) according to Dunkin's multiple range test.

3.6. Total Yield (ton.plastic house^{-1})

The results of Table 6 showed a significant effect of the cultivar on the total yield, as the plants of cultivar V2 were characterized by the highest yield of 8.673 tons.greenhouse^{-1}, while the plants of cultivar V3 recorded the lowest value of yield amounting to 5.320 tons.greenhouse^{-1}.

The results of the above table indicate that there are significant effects of boron fertilization treatments on the trait, as the plants treated with nanoparticle boron (F2) were characterized by the best yield of 8.963 tons.greenhouse^{-1}, while this value decreased to 3.767 tons.greenhouse^{-1} for non-fertilized plants (F0).

The results showed that there were significant effects of the interaction between the cultivar and the foliar fertilization with boron in the trait, as the treatment of (V2) plants with nanoparticle boron (V2F2) excelled with the highest yield of 10.91 tons plastic house^{-1}, while the non-fertilized (V3) plants recorded (V3F0). The lowest yield was 2.849 tons, plastic house^{-1}.

Table 6. The effect of cultivar and boron fertilization and their interaction on the total yield (ton.greenhouse^{-1}) for four genotypes of colored pepper*.

Boron fertilization Varieties	Comparison without composting (F_0)	Metallic boron 50 mg L^{-1} (F_1)	Nano boron 1 mg L^{-1} (F_2)	Nano boron 2 mg L^{-1} (F_3)	Varieties average
Carisma Improved (V_1)	3.154 h	5.895 f	8.324 bcd	6.986 cdef	6.089 BC
Golden California Wonder (V_2)	5.650 f	8.595 bc	10.91 a	9.541 ab	8.673 A
Big Red (V_3)	2.849 h	5.032 fg	6.914 cdef	9.487 def	5.320 C
Orange Sun (V_4)	3.417 gh	6.157 ef	9.708 ab	7.934 bcde	6.804 B
Boron fertilization averages	3.767 D	6.419 C	8.963 A	7.737 B	

* Means that take the same letter for each factor or for the interaction between them are not significantly different ($P \leq 0.05$) according to Dunkin's multiple range test.

4. Discussion

The results of the above tables show that there are significant differences between the genotypes in the characteristics of vegetative growth and yield. Orange sun was superior in chlorophyll content in leaves and plant height. While the Carisma Improved genotype was superior in the number of fruits and the Golden California Wonder genotype was superior in the total yield of the greenhouse and the size of the fruit. This difference may be attributed to the variation in the genetic content of these structures, as the genes of each structure were expressed in a different way and for each of the characteristics from the other structure under the same environmental conditions.

That is, genes and their interaction with environmental factors determine the physiological ability of genotypes to modify the products of carbon metabolism into food compounds that contribute to increasing growth in general, including vegetative and flowering growth[14;]. Spraying boron on the leaves increased the indicators of vegetative growth represented by the plant's high number of leaves. This may be attributed to the role of boron in building a root system with high efficiency in absorbing macro and micro nutrients and increasing their concentration inside the plant[15]and[16]. Boron has a positive role in activating and increasing the effectiveness of growth hormones, especially auxins and cytokinins[17]Boron also has an important role in the formation of proteins in plants through the formation of RNA, the work of membranes, nitrogen metabolism and photosynthesis, and the increase in the amount of carbohydrates manufactured in the leaves. And the proteins needed to build plant tissues[14]. It improves many physiological and biochemical processes by stimulating meristematic tissues, increasing cell division and elongation, and increasing the production and effectiveness of growth regulators, which reflects positively on vegetative indicators and their increase [18]. This is consistent with the findings of [11] that spraying boron on the leaves of sweet pepper led to an increase in plant height, number of branches, leaf area, dry weight compared to the comparison treatment.

what[19] concluded was that all treatments were superior to spraying with nutritional solutions containing boron, compared to the comparison treatment. These results also agree with the results of [20] When pepper was sprayed with boron, it improved the vegetative growth indicators. The reason for the superiority of the spray treatment with nanoparticles may also be due to the distinction of nanomaterial's, including nanoparticles, with rapid absorption and control of nutrient delivery to plant parts [21]. Which provides nutrients to the plant through its ease of absorption. Nano fertilizers also have various unique properties such as high charge density, high interaction, higher penetration power into plant tissues and heat resistance [22] .It was also found that it stimulates plant growth and is linked to environmental conditions, as it works to increase the plant's tolerance to unfavorable environmental conditions[5; 23].

The superiority of the plants treated with nano-boron at a concentration of 1 $mg.L^{-1}$ may be due to its direct effect on increasing and improving the vegetative growth of the plant. This in turn led to an improvement in the characteristics of flowering growth, which was reflected in an increase in the characteristics of the yield due to major roles of boron element in the division and elongation of root cells, especially in the developing part of it, and this has a great effect in increasing the absorption of nutrients necessary for plants, such as nitrogen and phosphorus, which have an encouraging effect on the vital and physiological activities of plant growth. As well as for its role in the formation of some nutrients necessary to increase the rate of plant growth. Boron has the ability to increase the efficiency of the plant in increasing the dry matter manufactured by the plant, and this positively affects the growth and production of the plant, which is reflected in one way or another on the plant yield[24]. Also, the superiority of plants treated with boron can be through its direct effect on the characteristics of flowering growth, such as the number of flowers and the percentage of knots. As it increases the fertilization process and the vitality of the female and intellectual parts, as well as its positive role in germination of pollen, the formation of the pollen tube, the speed of cell division after the process of contracting, and the increase in the number of fruits which in turn leads to an increase in yield components and then an increase in the total plant yield [25].

Conclusions

The investigation found that both the leaf chlorophyll content of orange sun (288.1 mg per 100 gm^{-1} fresh weight and the overall plant height were significantly higher than those of the control group (56.98 cm). The golden California wonder cultivar produced the most fruit per plant in a greenhouse, whereas the huge red and orange sun cultivars were the best at producing large fruits (89.87 and 89.20 cm^3).

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Effect of Spraying with Marvel Nutrient and Antioxidants on the Mineral Content of Apricot leaves Labib Cultivar

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Abstract. This study was carried out in one of the orchards of Kerbala city/ Al-Hussainiya district .Where 30 apricot trees of Labib cultivar were selected, each tree was considered as an independent experimental unit with three replications. , Foliar fertilizer by Marvel Nutrient solution at a concentrations (0,2) ml/L was the first factor ,and the second factor was antioxidants which represented by ascorbic acid and citric acid , at concentrations (500,1000) mg/L. for each tree. In addition water was sprayed as the control application. The results showed that there was a significant effect for experimental treatments on the content apricot trees leaves of the mineral elements (Nitrogen, phosphorus, potassium, zinc and iron), as the nutrient solution F2 and the antioxidant factor A2, S2 were superior. The interactions between factors F2*A2, F2* S2 (nutrient solution and antioxidants) was greater in giving the highest results levels of the content apricot trees leaves of the mineral elements (NPK, Fe and Zn).

Keywords. Apricot, Foliar fertilizer, Antioxidants, Nutrient solution.

1. Introduction

The Apricot *Prunus armenica* L. belongs to the subfamily *Prunoidae*, which belongs to the *Rosaceae* family. . Apricot trees are characterized by their rapid growth and fruiting [1]. Apricots are grown in temperate regions such as the Mediterranean countries, America, California in these places there are the greatest apricot farms in the world [2]. The nutritional importance of apricots is due to their high content of vitamin A and niacin, compared to other fruits, they are also an excellent source of sugar [3].

Apricots are grown in Iraq mostly in the central region, , as well as in the northen region , especially local varieties. It is not possibole to plant early varieties in cold regions for fear of spring freezes because the flowers open up early before vegetative growth [4].

Feeding the plant with mineral elements greatly affects the quantity of production and the quality of the fruit, as foliar fertilization has a major role in improving the growth of trees through the arrival for macronutrients such as nitrogen, phosphorus and potassium in a way that can absorbed by the leaves, as sufficient quantities of macronutrients are necessary for plant growth [5]. Nitrogen is one of the important and effective elements needed by the plant for it is effective role in the formation of cell protoplasm after water, and its ratio is about 2-4 % of the dry matter of the plant [6]. Phosphorus also has an active role in the vital processes of the plant such as the process of carbon metabolism. The

formation and demolition of carbohydrates and transfer of energy within the plant as well as it is role in accelerating flowering [7]. Potassium , plays a role in many physiological processes within the plant such as transfer of nutrients and salts, the formation of sugars and proteins , in addition to cell division [5].

Iron has an effective and essential role in the system of many enzymes involved in the process of respiration, including cytochrome oxidase, peroxidase, catalase, as it is important in transfer of electrons in oxidation and reduction reactions in addition it has a role in the nutritional metabolism of the cell [8]. It also has an essential role in the representation of amino acids and chloroplasts, as it helps in the construction of chlorophyll, although it does not enter into composition [9].

[10], indicated that treated apricot trees of the Canino cultivar in sandy soils with nutrients, it gave an increase production, especially treating it with boron. [11], found that when peach trees were sprayed with iron at a concentration of 300 mg/L. and pear trees, the Red variety of both trees containing iron at a rate of 250 mg/L alone or with zinc and manganese may increase leaf chlorophyll content and improve vegetative growth, flowering and fruiting. As for the role of antioxidants in improving of plant growth , ascorbic acid which is one of the antioxidants that have a role in stimulating and energizing the vegetative and fruiting growth for different fruit trees and its effect on the plant is like to effect of growth – active regulators [12,13] indicated that the role of different levels of ascorbic acid (0,25,250,750) mg/L. in the vegetative growth indicators of peach trees Early cultivar , the results showed a significant increase in in the number of flowers .

Citric acid is considered a non-enzymatic antioxidant , it has an effective role as it acts as a scavenger for free radicals resulting from the stresses that the plant is exposed to and which affect the disturbed nutritional transitions , many of studies and research have indicated the role of citric acid in the growth and production of some plant species , and it was indicated by [14] as he showed that foliar spraying with citric acid at a concentration of 300 mg / L on apple trees led to a significant increase in the number of flowers and fruiting branches and production. [15], showed that the importance of citric acid at a concentration of 2000 ppm in improving the growth characteristics of apple trees, (Anna variety), as it led to an increase in the number of flowers and improvement of fruit quality, and early production. Therefore the experiment aimed to investigate the role of foliar fertilization by nutrient solution Marvel and Antioxidants in the content of apricot tree leaves from mineral elements. .

2. Materials and Methods

This experiment was conducted on Apricot trees (Labib) in one of the orchards of Karbala Governorate / Al-Husseiniyah District during the growing season (2021-2022). The experiment was conducted on 30 trees, and each tree was treated as an independent experimental unit with three replications. The age of the trees was approximately 15 years old and the trees were in same size as possible , all planted by square method at distance (7*7) meter , grafted with apricot seed stocks that were reared on balls and were free of diseases. The all tested treatments were sprayed three times in the same trees each tree was sprayed until it reached the point of complete wetness . Before conducting the experiment, some service operations were carried out by removing the bushes, and the trees were distinguished by numbering (transaction numbers) randomly, in addition to washing the trees one day before the spraying process. . The experiment was designed in a randomized completely blocks design with three replicates, each consisted of one tree (RCBD). The statistical differences between the treatments were analyzed using the least significant difference (L.S.D.) at the probability 5%. Fertilizer treatments for foliar spray were prepared according the concentrations under study, and a spreader (Al-Zahi liquid detergent in the amount 0.1ml / L.) was added. The number of sprays, was 3 sprays from the beginning of March, and the period between one spraying and the other was 20 days. The spraying process was applied in the early morning.

Transaction concentrations and codes for each replicate:-

- F1Z0 = (0 ml /L. Marvel solution + 0 mg / L. antioxidants)
- F1A1 = (0 ml /L. Marvel solution + 500 mg / L. ascorbic acid)
- F1S1 = (0 ml /L. Marvel solution + 500 mg / L. citric acid)
- F1A2 = (0 ml /L. Marvel solution + 1000 mg / L. ascorbic acid)

- F1S2 = (0 ml /L. Marvel solution + 1000 mg / L. citric acid)
- F2Z0 = (2 ml /L. Marvel solution + 0 mg / L. antioxidants)
- F2A1 = (2 ml /L. Marvel solution + 500 mg / L. ascorbic acid)
- F2S2 = (2 ml /L. Marvel solution + 500 mg / L. Citric acid)
- F2A2 = (2 ml /L. Marvel solution + 1000 mg / L. Ascorbic acid)
- F2S2 = (2 ml /L. Marvel solution + 1000 mg / L. citric acid)

Table 1. The components of orchard soil.

Sample	pH	E.C	Sand	Silt	Mud	Soil texture
Soil	7.68	3.42	600 gm/kg soil	188 gm/kg soil	152 gm/kg soil	Sandy mixture

Table 2. The components of the solution (Marvel).

Components	The ratio
Ozot N_2O	2%
Phosphorous pentoxide P_2O_5	3%
Potassium oxide K_2O	15%
Chelated iron and a complementary set of amino acids	0.1%

2.1. Study Indications
The experiment included the following four treatments:

2.1.1. Nitrogen (% N)
Determination of nitrogen percentage: the nitrogen element was estimated according to the Keldal method using a device Micro-Kjeldal [16].

2.1.2. Phosphorus (%P)
Phosphorus was estimated by Spectrophotometer at wave length 882 nm [17].

2.1.3. Potassium (% K)
Which determination by flame meter [18].

2.1.4. Iron and Zinc
The concentrations both of Fe ,Zn (mg / gm dry matter) were estimated in the leaves using an atomic absorption spectro photometer according to the method mentioned in [19].

3. Results and Discussions

3.1. Leaves Content from Nitrogen (% N)
Table (3) shows that the significant effect of Marvel solution in concentration 2 ml/ l. (F2) on content of apricot leaves from nutrients, as the highest rate of nitrogen element in concentration 2 ml/l. (F2) was 2.091 % compared to the control treatment which gave the lowest rate it was 1.765%, as shown in Table (3). The significant effect of antioxidants, as a citric acid at a concentration 1000 gm/l. gave the highest rate of 2.250%. Meanwhile, the concentration 1000 gm/l. from ascorbic acid was less concentrated and recorded 1.778%. This may be caused by a role of foliar fertilization , including the important nutrients in vital processes , as the nitrogen element is important to the plant because it is included in the composition of most of the significant vital substances of the plant such as proteins , enzymes and nucleic acids (DNA ,RNA)[20] .

As for the interaction Table (3) showed that the significant effect of foliar fertilization (Marvel) with antioxidants (ascorbic and citric acid)in the content of apricot leaves of the nitrogen element, as it gave an interaction treatment (2 ml/l. marvel + 1000 mg/l. citric acid)(F2*S2) was highest rate 2.253% compared with the control treatment (0 ml/l. marvel + 0 mg/l. antioxidants) (F1*Z) which gave the lowest rate reached 1.493% .

Table 3. Effect of foliar fertilizer (Marvel) and antioxidants (citric and ascorbic acid) on the nitrogen content in apricot tree leaves.

Antioxidants Marvel	Control T. Z 0 mg /L.	Ascorbic acid T. A1 500 mg/L.	Ascorbic acid T. A2 1000 mg/L.	Citric acid T S1 500 mg /L.	Citric acid T S2 1000 mg /L.	Rate of Marvel solution
F1 0 ml / L.	1.496	1.557	1.890	1.640	2.247	1.765
F2 2 ml / L.	2.123	2.290	1.667	2.120	2.253	2.091
Rate of antioxidants	1.808	1.923	1.778	1.880	2.250	
L.S.D	Marval		antioxidants		interaction	
0.05	0.255		0.403		0.570	

3.2. Leaves Content from Phosphorus (%P)

From Table (4) the significant effect is clear of Marvel solution at concentration 2 ml/l. (F2) on content of apricot leaves from nutrients, where the highest rate of phosphorus element in concentration 2 ml/ L. (F2) was 0.251 % while the control treatment which gave the lowest rate it was 0.241%, as shown in Table (4) the significant effect of antioxidants, as a citric acid at a concentration of 1000 mg/l. gave the highest rate of 0.275% while the control treatment gave the lowest rate it was 0.217% .

This may be caused by a role of phosphorus element in the process of carbon metabolism and the construction and breakdown of carbohydrates and transfer energy. In addition, it is included in the composition of amino acids, energy-carrying compounds, and some enzymes [7] .

While the interaction treatments Table (4) shows the significant effect of Marvel solution with antioxidants (ascorbic and citric acid) in the content of apricot leaves of the phosphorus element, as it gave an interaction treatment (0 ml/l. Marvel + 1000 mg/l. citric acid) (F1*S2) highest rate was 0.276% compared with control treatment (0 ml/l. Marvel + 0 mg/l. antioxidants) (F1*Z0) which gave the lowest rate 0.217% .

Table 4. Effect of foliar fertilizer (marvel) and antioxidants (citric and ascorbic acid) on the phosphorus content in apricot tree leaves.

Antioxidants Marvel	Control T. Z 0 mg /L.	Ascorbic acid T. A1 500 mg/L.	Ascorbic acid T. A2 1000 mg/L.	Citric acid T S1 500 mg /L.	Citric acid T S2 1000 mg /L.	Rate of Marvel solution
F1 0 ml / L.	0.229	0.212	0.233	0.256	0.276	0.241
F2 2 ml / L.	0.205	0.267	0.263	0.247	0.274	0.251
Rate of antioxidants	0.217	0.239	0.248	0.251	0.275	
L.S.D	Marval		antioxidants		interaction	
0.05	0.003		0.004		0.006	

3.3. Leaves Content from Potassium (%K)

The significant effect is very clear from Table (5) of Marvel solution at concentration 2 ml/l. is the code given (F2) on content of apricot leaves from nutrients , where the highest rate of potassium element in concentration 2 ml/ L. (F2) was 1.999 % while the control treatment F1 which gave the lowest rate 1.643%, as shown in Table (5). The treatments of antioxidants has a significant effect, as a citric acid at a concentration of 1000 mg/l. gave the highest rate of 2.038% compared with ascorbic acid treatment A2, and control treatment Z0 those which gave the lowest rate they were 1.608% , 1.708% successively .

Perhaps the reason in this case revert to the role of foliar fertilizer which is very rich from micronutrients and its role in lots of physiologic process in to the plant such as transfer of salts of micronutrients and cell division [21] .

Further, the interaction treatments Table (5) shows the significant effect of Marvel solution with antioxidants (ascorbic and citric acid) in the content of apricot leaves of the potassium element, as it

gave an interaction treatment (2 ml/l. Marvel + 1000 mg/l. ascorbic acid) (F2*A2) highest rate was 2.130% compared with the interaction treatments F1A2 and F1Z0 which gave the lowest rate that were 1.370%, 1.473% successively .

Table 5. Effect of foliar fertilizer (Marvel) and antioxidants (citric and ascorbic acid) on the potassium content in apricot tree leaves.

Antioxidants Marvel	Control T. Z 0 mg /L.	Ascorbic acid T. A1 500 mg/L.	Ascorbic acid T. A2 1000 mg/L.	Citric acid T S1 500 mg /L.	Citric acid T S2 1000 mg /L.	Rate of Marvel solution
F1 0 ml / L.	1.473	1.810	1.370	1.477	2.087	1.643
F2 2 ml / L.	1.943	2.130	1.847	2.083	1.990	1.999
Rate of antioxidants	1.708	1.970	1.608	1.780	2.038	
L.S.D	Marval		antioxidants		interaction	
0.05	0.114		0.180		0.255	

3.4. Leaves Content from Iron (Fe mg /l.)

From Table (6) it was found that the significant effect of concentration 2 ml/l. is the given code (F2) of Marvel solution on content of apricot leaves from nutrients. Where the highest rate of iron element in concentration 2 ml/l. (F2) was 12.33 mg/l. while the control treatment F1 which gave the lowest rate 11.58 mg/l , as shown in Table (6). The antioxidants treatment has a significant effect, as a citric acid at a concentration of 1000 mg /l. (S2) gave the rate 12.65 mg/l. compared with control treatment Z0 which gave the lowest rate 10.44 mg/l.

This is consistent with what [22] found when they studyed the effect of foliar spraying with citric acid and nutrients on grapes, Red Globe cultivar where foliar spraying caused significant increase in most of the studied traits.

In addition the interaction treatments Table (6) explains the significant effect of Marvel solution with antioxidants (ascorbic and citric acid) in the content of apricot leaves of the iron element , as it gave an interaction treatment (2 ml/L. Marvel + 1000 mg / L. ascorbic acid) (F2*A2) highest rate 13.40 mg/L. compared with interaction treatment F1Z0 which gave the lowest rate of 8.81 mg/L.

Table 6. Effect the foliar fertilizer (Marvel) and antioxidants (citric and ascorbic acid) on iron content in apricot tree leaves.

Antioxidants Marvel	Control T. Z 0 mg /L.	Ascorbic acid T. A1 500 mg/L.	Ascorbic acid T. A2 1000 mg/L.	Citric acid T S1 500 mg /L.	Citric acid T S2 1000 mg /L.	Rate of Marvel solution
F1 0 ml / L.	8.81	12.11	12.25	11.59	13.13	11.58
F2 2 ml / L.	12.07	12.10	13.40	11.92	12.17	12.33
Rate of antioxidants	10.44	12.10	12.83	11.75	12.65	
L.S.D	Marval		antioxidants		interaction	
0.05	0.581		0.918		1.299	

3.5. Leaves Content from Zinc (Fe mg /l.)

The Table (7) shows the effect of foliar fertilization on the content of apricot leaves from zinc where it was concentration 2 ml/ L. is the given code (F2) of Marvel solution, where the highest rate of zinc element in concentration 2 ml/l (F2) was 26.72 mg/l. Compared with the control treatment F1 which gave the lowest rate it was 22.59 mg/l., as shown in Table (7). The antioxidants treatment have a significant effect but it wasn't a high increase, as citric acid at a concentration of 1000 mg/l. (S2) gave the rate of 27.58 mg/l. compared to control treatment Z0 that gave the lowest rate 21.82 mg/l.

The reason for this can be explained by the fact that citric acid can play the role of chelating substance for micronutrients that are sprayed on plants to facilitate their entry to the surface of the leaf to equalize the electric charge of leaf surfaces [23].

In addition to the interaction treatments between foliar fertilization and antioxidants, Table (7) indicated that there was no significant effect on the zinc content of leaves.

Table 7. Effect of foliar fertilizer (Marvel) and antioxidants (citric and ascorbic acid) on the zinc content in apricot tree leaves.

Antioxidants Marvel	**Control T. Z 0 mg /L.**	**Ascorbic acid T. A1 500 mg/L.**	**Ascorbic acid T. A2 1000 mg/L.**	**Citric acid T S1 500 mg /L.**	**Citric acid T S2 1000 mg /L.**	**Rate of Marvel solution**
F1 0 ml / L.	18.74	22.85	23.75	21.47	26.17	22.59
F2 2 ml / L.	24.90	27.03	24.79	27.87	28.99	26.72
Rate of antioxidants	21.82	24.94	24.27	24.67	27.58	
L.S.D	Marval		antioxidants		interaction	
0.05	1.979		3.129		N.S	

Conclusion

The nutrient solution F2 and antioxidant factor A2, S2 had the greatest influence on the mineral elements in apricot tree leaves (nitrogen, phosphorus, potassium, zinc, and iron). The interactions between components F2*A2, F2*S2 (nutrient solution and antioxidants) produced the maximum mineral element content in apricot tree leaves (NPK, Fe and Zn).

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Effect of Levels and Dates of Adding Amino Fertilizers (Ticamine Max) and Organic Liquid (Viviter) in the Mineral Content of Christi Thorn Seedlings

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Abstract. This experiment aimed to demonstrate the importance of adding levels and dates of fertilization with amino and organic liquid fertilizers in accelerating the growth of Christi thorn seedlings and their mineral content of nutrients to obtain strong, fast-growing seedlings. The study included three factors, two dates for adding fertilizers (every 15 days and every 30 days) and three concentrations (0 and 2 $ml.L^{-1}$ and 4 $ml.L^{-1}$) for each of the amino fertilizer (Tekamin Max) and the liquid organic fertilizer (Viviter). The most important results obtained showed that the concentration of 4 $ml.L^{-1}$ for the two fertilizers gave the highest increase Significant for the content of the leaves (nitrogen, phosphorus, potassium and protein). On the other hand, the dates of fertilizer application did not have any significant differences in the studied traits, except that the first date (addition every 15 days) was superior to the second date (addition every 30 days) in the nitrogen content of the leaves.

Keywords. Seedlings, Christi thorn, Fertilization, Amino acids, Liquid organic fertilization.

1. Introduction

Christi thorn is considered one of the evergreen fruits, which belongs to the family Rhamanaceae, which is one of the large plant families, which includes about 58 species, including three important genera, the most important of which is the genus Buckthorn. Cultivation is spread mostly in the tropical, subtropical and warm temperate regions of the world. The original habitat of buckthorn trees is the regions of southern Europe, the Himalayas, northern China, and it may be North Africa, Sudan, the Arabian Peninsula, and South America [1]. The fertilization process is one of the most important agricultural operations that take place in nurseries to encourage the growth of seedlings and obtain good-growing seedlings, especially in terms of the diameter of the main stem to facilitate the process of grafting them [2], The addition of organic fertilizers containing amino acids to fruit seedlings, including buckthorn, may lead to an increase in the soil content of nutrients ready for the plant and increase the strength of its vegetative and root growth, which is reflected positively on the content of the leaves of those seedlings of the mineral elements necessary for its growth [3].

The addition of organic fertilizers, including liquid ones, is of great importance in improving the growth of seedlings due to the fact that they contain some organic acids such as fulvic and humic acids, amino acids and other substances, which are characterized by their ease of use, low pollution to the environment and agricultural products, cheap prices, and their contribution to improving the

physical, chemical and vital characteristics of the soil as well as Its important role in increasing the uptake of nutrients such as nitrogen, phosphorus, potassium, iron, magnesium, copper, zinc, and others by plant roots, which results in increased vegetative and root growth [4,5]. Studies related to the effect of fertilizer feeding on vegetative growth and mineral content of Christi thorn seedlings are few, so this study comes in order to demonstrate the importance of adding fertilization with amino and organic liquid fertilizers in accelerating the growth of Christi thorn seedlings and improving their vegetative characteristics and mineral content to obtain strong seedlings, especially in terms of the diameter of the main stem To facilitate the process of budding as soon as possible.

2. Materials and Methods

The experiment was carried out during the 2022 growing season in the lath house on the one-year-old seedling of Christi thorn, which is approximately the same in strength, 25-30 cm in height, and the diameter of its main stem is 2-3 mm, which were planted in plastic sticks with a capacity of 5 kg filled with mixed soil. The experiment included the following factors:-

The first factor: two dates for adding fertilizers (every 15 days and every 30 days) with 6 additions for each of them, as the addition started from the beginning of the season at the beginning of April and continued until the beginning of September.

The second factor: the use of the amino fertilizer (Tekamin Max) containing (total amino acids 14.40%, free amino acids 13%, organic nitrogen 8.0%, organic matter 50.0%, pH 6.6) sprayed on shoots with three concentrations (0, 2,4 $ml.L^{-1}$).

The third factor: the use of liquid organic fertilizer (Viviter) containing (5% organic nitrogen, 7% potassium dioxide and 7% organic carbon) in addition to the soil at three levels (0, 2,4 $ml.L^{-1}$).

The experiment was designed using the randomized complete block design of factorial experiments (R.C.B.D) with three factors, three replications, and four seedlings for each experimental unit. Thus, the number of seedlings used in the experiment was (2 x 3 x 3 x 3 x 4 = 216), and the averages were compared using Dunkin's multiple limit test.

In mid-August, the following characteristics were measured:

- The concentration of nitrogen in the leaves, %.
- The concentration of phosphorus in the leaves, %.
- Potassium concentration in leaves %.
- Total protein concentration in leaves %.

3. Results and Discussion

The results of Table (1) indicate that the concentrations of the amino fertilizer (Tecamine Max) induced a significant increase in the nitrogen content of the leaves, as the treatment of 4 $ml.L^{-1}$ recorded the highest values and significantly outperformed the rest of the concentrations, and it also gave the highest concentration of liquid organic fertilizer (Viviter) 4 $ml.L^{-1}$ significantly increased in this trait compared to the rest of the concentrations, while the first date (addition every 15 days) was superior to the second date (addition every 30 days) in the values of the nitrogen content of the leaves.The results of the same table related to the interaction between the dates and the amino fertilizer showed that the treatment of the two-way interaction between the concentration of 4 $ml.L^{-1}$ of the amino fertilizer added at the first date (every 15 days) gave the highest significant increase in the values of the nitrogen content of the leaves and recorded a significant superiority over all the interactions. Binary, as is the case with regard to the overlap between dates and concentrations of liquid organic fertilizer, as the treatment of the bilateral overlap between the treatment of 4 $ml.L^{-1}$ of liquid organic fertilizer and the addition (every 15 days) achieved the highest significant increase in the values of this characteristic, superior to all other interactions, as for the bilateral overlap Between the concentrations of each of the amino fertilizer and the liquid organic fertilizer, it was found that the intervention treatment between the concentration of 4 $ml.L^{-1}$ for both fertilizers achieved the highest significant values of the values of the nitrogen content of the leaves, and significantly outperformed all binary interactions, As for the triple interactions between the study factors, it is noted that the treatment of the triple interaction between a concentration of 4 $ml.l^{-1}$ of amino fertilizer and liquid organic fertilizer added every 15 days achieved the highest significant values of the nitrogen content

of the leaves and significantly outperformed all the triple interactions except for the treatment of the triple interaction between the same concentration of fertilizers added every 30 days.

Table 1. The effect of the levels and dates of addition of amino fertilizer (Tecmine Max) and organic liquid (VivIter) and the interaction between them on the content of leaves of nitrogen Christi thorn seedling.

Liquid Organic Fertilizer Concentrations (Viviter)						
Addition dates	**Amino fertilizers Concentrations Tecmine Max**	**Control**	**2 ml.L^{-1}**	**4 ml.L^{-1}**	**Average addition dates**	
First date after 15 days	Control	1.60e-f	1.96 h-f	2.10 j-h	Date 1	2.37a
	2ml.L^{1-}	1.89h g	1.93h g	2.46 e-f	Date 2	2.21 b
	4ml.L^{1-}	2.92b-c	3.02 b-c	3.48a	Average concentrations of amino fertilizer Tecmine Max	
Second date after 30 days	Control	1.36j	1.76j g	1.87h g	1.77c	
	ml 2.L^{1-}	2.01h-f	2.20 e-f	2.11 e-f	2.10b	
	ml 4.L^{1-}	2.53e-c	2.92 b-c	3.18a b	3.01a	
interaction between dates and organic fertilizer	First date after 15 days	2.13b c	2.30b	2.68a	interaction between dates and the amino fertilizer	
	Second date after 30 days	1.97c	2.29b	2.38b	First date after 15 days	Second date after 30 days
interaction between amino fertilizer and organic fertilizer	Control	1.48e	1.86d	1.98c d	1.89c d	1.66d
	2ml.L^{1-}	1.95c d	2.07c d	2.28c	2.09c	2.10c
	4ml.L^{1-}	2.73b	2.97b	3.33a	3.14a	2.88b
Average concentrations of liquid organic fertilizer		2.05c	2.30b	2.53a		

*The averages followed by different letters indicate significant differences between them according to the Duncan test at the level of 5%.

The results mentioned in Table (2) confirmed that the concentrations of the amino fertilizer Ticamine Max recorded a significant superiority compared to the comparison treatment with the values of the characteristic of the leaf content of phosphorous, especially the concentration 4 ml.L^{-1}, which recorded the highest significant values for this characteristic, and the effect of the concentrations of the liquid organic fertilizer Viviter was similar to the concentrations of Amino fertilizer, as the organic fertilizer concentrations achieved a significant superiority with the values of this characteristic, especially the concentration 4 ml.L^{-1}, which gave the highest values compared to the comparison treatment. On the other hand, it is noted that the application times did not have any significant effect on the phosphorus values in the leaves.

As it is clear from the results of the same table regarding the binary interaction between the dates of application and the concentrations of the amino fertilizer, the treatment of the interaction between the concentrations of 4 ml.L^{-1} and the one added every 30 days gave the highest significant value for this characteristic, but it did not outperform the two treatments of the binary interaction between the comparison treatment of the amino fertilizer and the one added every 15 and 30 days, and the same applies to the treatment of the bilateral interaction between the concentrations of liquid organic fertilizer and the dates of application, as the highest significant value was the result of the interaction

between the concentration of 2 ml.L^{-1} and that added every 15 days, but it was only superior to the two treatments of the bilateral interaction between the comparison treatment of organic fertilizer The liquid and the additive every 15 and 30 days. As for the bilateral interaction between the amino fertilizer and the liquid organic fertilizer, we find that all the binary interactions did not have any significant differences, except for the treatment of the bilateral interaction between the comparison treatment and for both fertilizers, as the lowest significant values for this characteristic were recorded, while the highest significant value was recorded. As a result of the bilateral interference between the same concentration (4ml.L^{-1}) and both fertilizers. The results of the table also indicate that the highest significant value of the characteristic of the leaf content of phosphorous for the triple interaction between the study factors was due to the triple interaction between the treatment of (4 ml.L^{-1} of amino fertilizer + 2 ml.L^{-1} of liquid organic fertilizer, which was added every 30 days day), but it did not significantly outweigh only the two treatments of interaction between (comparison for all fertilizers, which were added every 15 and 30 days).

Table 2. The effect of the levels and dates of addition of amino fertilizer (Tecmine Max) and liquid organic (Viviter) and the interaction between them in the content of leaves of phosphorus Christi thorn seedling.

Liquid Organic Fertilizer Concentrations (Viveter)						
Addition dates	**Amino fertilizers Concentrations Tecmine Max**	**Control treatment**	**2ml.L $^{1-}$**	**4ml.L $^{1-}$**	**Average addition dates**	
First date after 15 days	Control	0.283 d	0.413 a b	0.393 a b	Date 1	0.395 a
	2ml.L $^{1-}$	0.412 a b	0.403 a b	0.414 a b	Date 2	0.385 a
	4ml.L $^{1-}$	0.403 a b	0.413 a b	0.418 a b	Average concentrations of amino fertilizer Tecmine Max	
Second date after 30 days	Control	0.304 c d	0.350 b c	0.380 a b	0.354 b	
	ml 2.L $^{1-}$	0.374 a b	0.399 a b	0.403 a b	0.401 a	
	ml 4.L $^{1-}$	0.410 a b	0.423 a	0.420 a b	0.414 a	
interaction between dates and organic fertilizer	First date after 15 days	0.366 b c	0.410 a	0.408 a	interaction between dates and the amino fertilizer	
	Second date after 30 days	0.362 c	0.391 a c	0.401 a b	First date after 15 days	Second date after 30 days
interaction between amino fertilizer and organic fertilizer	Control	0.293 b	0.382 a	0.386 a	0.363b c	0.345 c
	2ml.L $^{1-}$	0.393 a	0.401 a	0.408 a	0.410 a	0.392a b
	4ml.L $^{1-}$	0.406 a	0.418 a	0.419 a	0.411 a	0.417 a
Average concentrations of liquid organic fertilizer		0.364 b	0.400 a	0.404 a		

*The averages followed by different letters indicate significant differences between them according to the Duncan test at the level of 5%.

It is noted from the results of Table (3) that the concentration of 4 ml.L^{-1} of the amino fertilizer caused a significant increase in the potassium content of the leaves compared to the rest of the concentrations, especially the comparison treatment, which gave the lowest value for the mentioned trait. The

concentrations of liquid organic fertilizer had a positive effect on increasing the potassium content. The leaves contain the element potassium, especially when adding the concentration 4 ml.L^{-1} of it, as it gave the highest significant value for this characteristic, and the times of adding fertilizers did not have any significant effect on the potassium content of the leaves. As for the bilateral interactions, we find that the two interactions between adding the concentration 4 ml.L^{-1} of the amino fertilizer to both dates (every 15 and 30 days) gave the highest significant increase in this capacity compared to the rest of the bilateral interactions between the two factors, and the same applies to the interaction between fertilizer concentrations. Liquid organic matter and application times, as the highest significant value for this characteristic was due to the bilateral interaction between adding the concentration 4 ml.L-1 of liquid organic fertilizer to both dates (every 15 and 30 days). As for the bilateral interaction between amino fertilizer concentrations and liquid organic fertilizer, we find The interaction between the same concentration (4 ml.L^{-1}) and both fertilizers produced the highest significant difference in the potassium content of the leaves and significantly outperformed most of the interactions. The results of the same table with regard to the triple interaction between the factors of the study indicate that the interaction treatment between (4 ml.L^{-1} of amino fertilizer + 4 ml.L^{-1} of liquid organic fertilizer added every 15 days) caused a significant increase in this capacity compared to the rest. Other triple interactions.

Table 3. The effect of the levels and dates of addition of amino fertilizer (Tecmine Max) and liquid organic (Viviter) and the interaction between them in the content of leaves of potassium Christi thorn seedling.

Liquid Organic Fertilizer Concentrations (Viviter)						
Addition dates	**Amino fertilizers Concentrations Tecmine Max**	**Control**	**2ml.L $^{1-}$**	**4ml.L $^{1-}$**	**Average addition dates**	
First date after 15 days	Control	1.03g	1.24g-f	1.40e-f	Date 1	1.614 a
	2ml.L $^{1-}$	1.46c-f	1.85a b	1.81a b	2 Date	1.640 a
	4ml.L $^{1-}$	1.89a	1.80a b	2.02a	Average concentrations of amino fertilizer Tecmine Max	
Second date after 30 days	Control	1.01g	1.14g	1.53b-c	1.22c	
	ml 2.L $^{1-}$	1.77b-c	1.82a b	1.69b-c	1.73b	
	ml 4.L $^{1-}$	1.90a	1.91a	1.97a	1.91a	
interaction between dates and organic fertilizer	First date after 15 days	1.46b	1.63a b	1.74a	interaction between dates and the amino fertilizer	
	Second date after 30 days	1.56a b	1.62 a b	1.73a	First date after 15 days	Second date after 30 days
interaction between amino fertilizer and organic fertilizer	Control	1.02e	1.19e	1.46d	1.22c	1.22c
	2ml.L $^{1-}$	1.61c d	1.83a-c	1.75b c	1.71b	1.76a b
	4ml.L $^{1-}$	1.90a b	1.85a b	1.99a	1.90a	1.93a
Average concentrations of liquid organic fertilizer		1.51b	1.62a b	1.74 a		

*The averages followed by different letters indicate significant differences between them according to the Duncan test at the level of 5%.

The results shown in Table (4) indicate that the fertilizer treatment 4 ml.l^{-1} of the amino fertilizer was significantly superior to the rest of the treatments, especially the comparison of leaf protein content values, as it achieved the highest values, and the same applies to the fertilizer treatment 4 ml.l^{-1} of organic fertilizer. The liquid, while the dates of adding fertilizers did not have any significant effect on the values of this trait. As for the two-factor interaction coefficients, we find that the interaction between the treatment of 4 ml.L^{-1} of the amino fertilizer, which was added every 15 and 30 days, had a

significant effect compared to the rest of the binary interactions and achieved the highest values for this characteristic. It is also noted that the two-interference treatment The concentration of 4 ml.L^{-1} of the organic fertilizer, which was added every 15 days, caused a significant increase in the values of the protein content of the leaves and significantly outperformed all the binary interactions between the two factors. As for the effect of the bilateral interaction between the concentrations of the amino fertilizer and the organic fertilizer, the results of the table showed In the same way, the two interactions between the same concentration of two fertilizers (4ml.L^{-1}) recorded the highest values and were significantly superior to all binary interactions. The results of the same table also show that the triple interaction between the factors of the study had a significant effect on the values of the protein content of leaves, as the highest significant value was recorded as a result of the triple interaction between (4 ml.L^{-1} of amino fertilizer and liquid organic fertilizer when added every 15 days).

Table 4. The effect of the levels and dates of addition of amino fertilizer (Tecmine Max) and liquid organic (Viviter) and the interaction between them on the content of leaves of protein Christi thorn seedling.

		Liquid Organic Fertilizer Concentrations (Viviter)				
Addition dates	**Amino fertilizers Concentrations Tecmine Max**	**Control**	**2ml.L $^{1-}$**	**4ml.L $^{1-}$**	**Average addition dates**	
First date after 15 days	Control	8.57h	12.27g f	13.16e-f	Date 1	14.69a
	2ml.L $^{1-}$	11.81g	12.10g	15.39e-f	2 Date	13.85 a
	4ml.L $^{1-}$	18.26b-c	18.89b-c	21.78a	Average concentrations of amino fertilizer Tecmine Max	
Second date after 30 days	Control	8.51h	10.99h g	11.68g	10.86c	
	ml 2.L $^{1-}$	12.56g f	13.78e-f	13.18e-f	13.13b	
	ml 4.L $^{1-}$	15.84e-c	18.28b-c	19.87a b	18.82a	
interaction between dates and organic fertilizer	First date after 15 days	12.88c d	14.42b c	16.78a	interaction between dates and the amino fertilizer	
	Second date after 30 days	12.30d	14.35b c	14.91b	First date after 15 days	Second date after 30 days
interaction between amino fertilizer and organic fertilizer	Control	8.54e	11.63d	12.42c d	11.33c	10.39c
	2ml.L $^{1-}$	12.18c d	12.94c d	14.28c	13.10b	13.17b
	4ml.L $^{1-}$	17.05b	18.59b	20.83a	19.64a	18.00a
Average concentrations of liquid organic fertilizer		12.59c	14.38b	15.84a		

*The averages followed by different letters indicate significant differences between them according to the Duncan test at the level of 5%.

The superiority of the concentration of 4 ml.L^{-1} of each of the amino fertilizer Tekamin Max and the liquid organic fertilizer viviter in the leaf content of nutrients (nitrogen, phosphorus, potassium and protein) came in agreement with the results of [6,7], that the addition of biofertilizers led to a significant increase in the content of olive and pomello leaves of nutrients, as it coincided with the results of [8,9], that the addition of liquid organic fertilizers caused a significant increase in the content of leaves Christi thorn and grapefruit are nutrients. These results can be explained by the fact that the liquid organic and amino fertilizers contain amino acids in their composition, in which nitrogen is included in their composition. Therefore, the addition of this fertilizer led to an increase in the absorption of nutrients from the soil and their transfer to the top, and these elements were liberated to

accumulate in the leaves, and their concentration increased. of organic matter, which works to improve the soil's chemical, biological and physical properties by disintegrating soil granules, improving its aeration, increasing its ability to retain water and its content of nutrients, and stimulating beneficial microorganisms in the soil, which leads to an increase in the readiness of these elements and their absorption by seedlings and their accumulation in the leaves, thus increasing their concentration [10-12].

We conclude from the results of this study that the addition of each of the liquid organic and amino fertilizers had an important role in increasing the nutrients content of the leaves, especially when adding the concentration 4 $ml.l^{-1}$ each.

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Effect of Cultivars, Apical Pinching and Copper Nano-Fertilizer on 1- Characteristics of Vegetative Growth of Broad Bean (*Vicia faba* L.)

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Abstract. Research was conducted at the vegetable field of the University of Mosul's Department of Horticulture and Landscape Engineering (in the College of Agriculture and Forestry) (2021). The experiment included a study of three factors, the first of which was the use of three different bean sprout cultivars (Spanish, American, and Dutch), the second of which was the condition of apical pinching (apical pinching and without pinching), and the third of which was the use of copper Nano-fertilizer at three different concentrations (zero, one, and 1.5 gm L-1) (2 x 3 x 3). Field research was conducted utilizing a factorial experiment with split-plots in R.C.B.D. Varieties were planted in the main plots, and the correlation between pinching and copper Nano-fertilizer concentrations was observed in the sub-plots. The Duncan multiple range test was used to compare the means of the groups, and a significance threshold of 5% was used for the study. Here's a rundown of the A- The significant effect of the plants of the three cultivars varied in the characteristics of the vegetative growth, as the plants of the Spanish and American cultivars were significantly superior in plant height and percentage of dry matter, while the plants of the Dutch and Spanish cultivars excelled in the number of branches, while no significant differences were observed between the cultivars in terms of chlorophyll and leaf area. B- The treatment of apical pinching excelled in terms of the number of branches and leaf area compared to the treatment of not pinching. C- The use of copper Nano-fertilizer in two concentrations resulted in significant effects on all vegetative growth traits compared to comparison plants. D- Most of the effects of the binary and triple interaction of the studied factors were consistent with the single effect of each factor for most of the previous characteristics.

Keywords. Apical Pinching, Vicia faba L., Copper.

1. Introduction

The broad bean *Vicia faba* L. according to its scientific name, is a kind of legume (Fabaceae). The leading producer is China, followed by Ethiopia. It is ranked fourth among grain crops in terms of significance, behind chickpeas, lentils, and peas, and helps provide food security in certain regions. A 100-gram serving of fresh green pods and seeds provides 72.6 grams of water, 0.73 grams of fat, 17.63 grams of carbohydrates, 7.5 grams of fiber, 1.55 milligrams of iron, 1.55 milligrams of magnesium, 129 milligrams of phosphorus, 332 milligrams of potassium, 3.7 milligrams of vitamin C, 0.133

milligrams of thiamine, 0.104 milligrams of vitamin B, 17 milligrams of vitamin These numbers change depending on the kind of plant and its growing circumstances. Dried seeds have a protein content of 15-21%. Choosing the right cultivar for each environment is fundamental to the success of the agricultural process and achieving high production in quantity and quality, and so plant breeders and producers constantly strive to introduce new varieties of high production and adaptation. In accordance with regional circumstances, which provide the bedrock for the spread of the crop's agricultural area [4].

In recent years, the world has tended to study several modern technologies in the agricultural field, especially the study of the possibility of using nanotechnology to improve the efficiency of fertilizer use towards manufacturing and developing what is known as Nano-fertilizers [5,6]. The use of Nano-fertilizers leads to an increase in plant productivity by increasing the absorption of nutrients [7,8]. It also significantly improves the growth of roots and seedlings and enhances the metabolism of the plant [9]. Recently, the idea of using fertilizers manufactured using nanotechnology has been adopted, which it is hoped will solve the problem of excessive use of conventional fertilizers [10]. Many studies indicated that there is strong competition for nutrients between the reproductive and vegetative parts and it is necessary to find some operations that aim to provide nutrients for the reproductive parts for the purpose of increasing production and improving quality ,the most important of these operations is pinching the growing tip of the main stem and side branches, as this process achieves the perfect balance between the vegetative and reproductive system and contributes to giving a large crop of good quality [11].

1.1. Research Aims

Evaluation and behavioral study of three cultivars of broad bean under the conditions of Nineveh Governorate, in order to choose which one is best suitable for cultivation under the conditions of the region, study the apical pinching in order to raise and improve Characteristics of vegetative growth of broad bean, and to find the best concentration of Nano-copper fertilizer that plants respond to and affect the vegetative growth characteristics, and to find the best triple overlap between cultivars , planting distances and boron Nano fertilizer concentrations.

2. Materials and Methods

Throughout the 2021 growing season, researchers from the University of Mosul's College of Agriculture and Forestry conducted an experiment on a vegetable field located inside the Tourist forest area. First, the effects of three different broad bean seed cultivars (Spanish, American, and Dutch), second, the effects of two different apical pinching conditions (with and without pinching), and third, the effects of three different doses of copper nano-fertilizer, were investigated (0, 1, and 1.5 gm L^{-1}), After 30 days of germination, at 15 day intervals throughout the second and third phases of plant development, Nano-fertilizer was applied to the plants. This study used a factorial design with split plots in R.C.B.D., with variety in the main plots and the correlation between pinching and copper Nano-fertilizer concentrations in the sub-plots, for a total of 18 treatments and 3 replicates. The ground was subdivided to form a number of 1.8 m long by 75 cm broad experimental units, four of which are ridges. The experimental unit covered 5.40 square meters, and on 9/12/2021, 24 seeds were sown there at 30-centimeter intervals along the top third and along one side of the ridges. The growing and harvesting of broad beans were carried out organically and according to established protocols. All treatments received fertilization with 100 kilograms of urea per hectare (46 percent nitrogen), 100 kilograms of potassium sulphate per hectare (48 percent potassium dioxide), 150 kilograms of triple superphosphate per hectare (45 percent phosphate dioxide), and 150 kilograms of potassium dioxide per hectare (per hectare) over the course of three separate applications: the first, 15 days after planting, included the application of all of the phosphate fertilizer, the second, after field emergence was complete, included The data was analyzed statistically using Duncan's test procedure at the 0.01-0.05 level of significance [12].

2.1. Studied Traits

- Total chlorophyll content in leaves.
- Plant height.
- Branches number.
- Leaf area.

3. Results

3.1. Total Chlorophyll Content in Leaves (mg ml suspension^{-1})

The results of Table (1) indicate that the three bean cultivars under study (Spanish, American and Dutch) did not differ significantly among them in the leaves' content of total chlorophyll. This trait was not affected significantly according to the condition of the pinching. As for the copper Nano-fertilizer, the results indicate that there is a significant increase in this characteristic as a result of the treatment with both concentrations of 1 and 1.5 gm L^{-1} of Nano-copper compared to the control treatment. The results of the bilateral interaction between the cultivars and the condition of the pinching showed the highest significant values in the leaves content of total chlorophyll, which were recorded in the case of the two treatments of the bilateral interaction between the Spanish and Dutch cultivar plants with the apical pinching treatment, as the leaf content of total chlorophyll was 24.45 and 24.42 mg ml suspension^{-1}, respectively, for each treatment. Thus, the two treatments of these two interactions were significantly superior to the treatment of the interaction between plants of the Spanish variety without pinching, as the total chlorophyll content of the leaves decreased in this treatment to 22.97 mg ml of suspension^{-1}. The results of the bilateral interaction between the cultivars and the copper Nano-fertilizer indicate that the highest significant value in the leaf content of total chlorophyll amounted to 25.89 mg ml suspension^{-1}, which was obtained in the Dutch cultivar plants, and the use of copper Nano-fertilizer at a concentration of 1 g.L^{-1}, and thus this treatment was significantly superior compared to all comparison treatments of the three cultivars, it was also significantly superior to the interaction treatment between the Dutch cultivar when using the concentration of 1.5 mg ml suspension^{-1} of this fertilizer. As shown by the results of the bilateral interaction between the pinching case and the use of copper Nano-fertilizer, all of the two-interference treatments resulted in a significant increase in this characteristic compared to the treatment without pinching and without using copper Nano-fertilizer, in which the leaf content of total chlorophyll decreased to 20.39 mg ml suspension^{-1}. A 1 mg ml Suspension^{-1} concentration of copper Nano-fertilizer resulted in a 25.13 mg ml Suspension^{-1} chlorophyll content in plants that were not pinched. According to the study's findings, total chlorophyll content in the leaves of Dutch variety plants increased by 26.63 mg ml suspension^{-1} when subjected to pinching and a copper Nano-fertilizer concentration of 1 g.L^{1}. This finding is based on the triple interaction coefficients between the three factors of the study.

Table 1. Effect of cultivars, Case of pinching, Nano-copper fertilizer and the interaction between them on Total chlorophyll content in leaves (mg ml suspension^{-1}).

Cultivars	Case of pinching	Concentration of Nano-copper fertilizer			Cultivars X Case of pinching	Average effect of Cultivars
		0	1	1.5		
Spanish	Without pinching	18.44 e	25.06 a-c	24.51 a-d	22.67 b	23.60 a
	pinching	23.94 a-d	25.63 ab	24.04 a-d	24.54 a	
American	Without pinching	21.82 cd	25.17 a-c	25.12 a-c	24.04 ab	23.81 a
	pinching	23.49 a-d	22.76 b-d	24.49 a-d	23.58 ab	

Cultivars	Case of pinching	Concentration of Nano-copper fertilizer			Cultivars X Case of pinching	Average effect of Cultivars
		0	1	1.5		
Dutch	Without pinching	20.92 de	25.15 a-c	24.09 a-d	23.39 ab	23.91 a
	pinching	24.17 a-d	26.63 a	22.51 b-d	24.44 a	
Cultivars X Nano- copper fertilizer	Spanish	21.19 d	25.34 ab	24.28 a-c		
	American	22.66 cd	23.96 a-c	24.81 a-c	Average effect of pinching case	
	Dutch	22.54 cd	25.89 a	23.30 b-d		
Case of pinching X Nano- copper fertilizer	Without pinching	20.39 b	25.13 a	24.58 a	23.37 a	
	pinching	23.87 a	25.01 a	23.68 a	24.19 a	
Average effect of Nano-copper fertilizer		22.13 b	25.07 a	24.13 a		

Averages that share the same letter for each factor and each interaction do not differ significantly between them according to Duncan's polynomial test at the probability level ($P < 0.05$).

3.2. Plant Height (cm plant^{-1})

According to the data shown in Tables (2), there was no discernible difference in plant height between the Spanish and American cultivars, and the American cultivar was clearly superior than the Dutch cultivar. There was no discernable difference between the apical pinching and non-pinching groups with regard to this characteristic. While plants treated with copper nano-fertilizer at 1 mg L^{-1} grew noticeably taller than those not given any fertilizer as all. Plant height attained a maximum of 106.29 cm plant^{-1} in the case of the Spanish cultivar plants subjected to the pinching treatment, suggesting a bilateral interaction between the cultivars and the pinching condition. Bilateral interaction findings between cultivars and Nano-copper fertilizer showed that plants of the American variety grown with 1 g L^{-1} of Nano-copper fertilizer recorded the greatest value in plant height (108.63 cm). Plant^{-1}. Whereas the minimum height of a plant was 92.20 cm Using a concentration of 1.5 g.L^{-1} of copper Nano-fertilizer, plants of a Dutch cultivar showed a high level of plant-1. The maximum value in plant height was obtained in the case of the interaction between the pinching treatment and the concentration of 1 mg L^{-1} of copper, while the lowest value was found in the case of the interaction between the pinching treatment and the concentration of 94.93 cm. As a result of not pinching the plants and without using copper Nano-fertilizer, Plant^{-1} developed. The highest value in plant height was 109.67 cm plant^{-1} and was found in the American cultivar plants under pinching and concentration 1 g L^{-1} of copper Nano-fertilizer, while the lowest value in plant height was 87.93 cm plant^{-1} and was recorded in the Spanish cultivar plants without pinching in the comparison plants.

Table 2. Effect of cultivars, Case of pinching, Nano-copper fertilizer and the interaction between them on Plant height (cm plant^{-1}).

Cultivars	Case of pinching	Concentration of Nano- copper fertilizer			Cultivars X Case of pinching	Average effect of Cultivars
		0	1	1.5		
Spanish	Without pinching	87.93 f	103.53 a-e	92.87 ef	94.78 b	100.53 a
	pinching	102.80 a-e	107.07 a-d	109.00 ab	106.29 a	
American	Without pinching	101.53 a-f	107.60 a-c	107.87 a-c	105.67 a	104.63 a
	pinching	98.47 a-f	109.67 a	102.67 a-e	103.60 a	
Dutch	Without pinching	95.33 b-f	92.27 ef	94.27 c-f	93.96 b	92.98 b
	pinching	93.47 d-f	92.40 ef	90.13 ef	92.00 b	
Cultivars X Nano- copper fertilizer	Spanish	95.37 b	105.30 a	100.93 ab		
	American	100.00 ab	108.63 a	105.27 a	Average effect of pinching case	
	Dutch	94.40 b	92.33 b	92.20 b		
Case of pinching X Nano- copper fertilizer	Without pinching	94.93 b	101.13 ab	98.33 ab	98.13 a	
	pinching	98.24 ab	103.04 a	100.60 ab	100.63 a	
Average effect of Nano-copper fertilizer		96.59 b	102.09 a	99.47 ab		

Averages that share the same letter for each factor and each interaction do not differ significantly between them according to Duncan's polynomial test at the probability level ($P < 0.05$).

3.3. Branches Number (branch plant^{-1})

According to Table (3), there was no statistically significant difference in branching between the Spanish and Dutch cultivars, but the Dutch cultivars were clearly superior to their American counterparts. The table shows that plants subjected to the pinching treatment produced much more branches than those that were not subjected to the pinching treatment. The findings for the Nano-copper fertilizer show that a concentration of 1.5 g.L^{-1} of copper nanoparticles significantly improves this trait compared to the control treatment and the treatment with a concentration of 1 g.L^{-1} of copper nanoparticles. The number of branches reached a statistically significant 9.73 in the event of interact between plants of the Dutch cultivar and the pinching treatment, according to the findings of the bilateral overlap between cultivars and the condition of pinching. The number of branches reached 7.76 in the two-way treatment comparing the American cultivar with the non-earring treatment, indicating some value in this characteristic. Plant^{-1}. The highest significant value in the number of branches was 11.57 branches plant-1 in the Dutch cultivar plants when using Nano-copper fertilizer at a concentration of 1.5 g.L^{-1}, while the lowest number of branches was 3.70 branch plant-1 in the comparison plants of the Spanish cultivar. The results of the bilateral interaction between the pinching case and the Nano-copper fertilizer show that this characteristic was significantly increased in the treatments of the bilateral interaction between the pinching process and the use of copper Nano-

fertilizer at a concentration of 1.5 g.L^{-1} compared to all other interaction treatments, as the number of branches of this treatment was superior Significantly on all treatments 10.61 branch.plant^{-1}, while the t-value was significantly lower. The number of branches increased by a maximum of 12.33 branch plant-1 in Dutch cultivar plants when using pinching and a concentration of 1.5 g.L^{-1} of Nano-copper fertilizer, according to the results of the triple interaction between the studied factors (varieties, condition of pinching, and Nano-copper fertilizer). The lowest value in this attribute was obtained in Spanish cultivar plants without pinching, making this treatment considerably superior to most of the treatments in this overlap.

Table 3. Effect of cultivars, Case of pinching, Nano-copper fertilizer and the interaction between them on Branches number (branch plant^{-1}).

Cultivars	Case of pinching	Concentration of Nano- copper fertilizer			Cultivars X Case of pinching	Average effect of Cultivars
		0	1	1.5		
Spanish	Without pinching	6.20 g	8.53 c-f	9.07 b-d	7.93 c	8.44 ab
	pinching	7.47 d-g	9.27 b-d	10.10 bc	8.94 b	
American	Without pinching	6.60 fg	8.07 d-g	8.60 c-f	7.76 c	8.13 b
	pinching	7.40 d-g	8.73 c-e	9.40 b-d	8.51 bc	
Dutch	Without pinching	6.40 g	6.90 e-g	10.80 ab	8.03 c	8.88 a
	pinching	7.87 d-g	9.00 b-d	12.33 a	9.73 a	
Cultivars X Nano- copper fertilizer	Spanish	6.83 e	8.90 bc	9.58 b		
	American	7.00 e	8.40 b-d	9.00 bc	Average effect of pinching case	
	Dutch	7.13 de	7.95 c-e	11.57 a		
Case of pinching X Nano- copper fertilizer	Without pinching	6.40 d	7.83 c	9.49 b	7.91 b	
	pinching	7.58 c	9.00 b	10.61 a	9.91 a	
Average effect of Nano-copper fertilizer		6.99 c	8.42 b	10.05 a		

Averages that share the same letter for each factor and each interaction do not differ significantly between them according to Duncan's polynomial test at the probability level ($P < 0.05$).

3.4. Leaf Area (cm^2 plant^{-1})

The results of Table (4) indicate that the plants of the three cultivars under study did not differ significantly among themselves in the leaf area of each plant. As for the effect of the condition of pinching on the leaf area, the results presented in the table show that the treatment of pinching in the study site is superior to the treatment of no pinching. As for the effect of copper Nano-fertilizer, the results indicate that there is a significant increase in this characteristic as a result of the treatment with a concentration of 1.5 g.L^{-1} of Nano-copper compared to the control treatment and the treatment with a concentration of 1 g.L^{-1} of copper Nano-fertilizer, in addition to the superiority of the treatment of the concentration of 1 g.L^{-1} of copper Nano-fertilizer over the control treatment. As for the bilateral

overlap between cultivars and the condition of pinching, the results indicate that the highest value in plant height amounted to 10958.3 cm ^{2} plant^{-1} recorded in the case of Dutch cultivar plants with the treatment of pinching, and this overlap treatment was significantly superior to the non-pinching treatment for all cultivars. The results of the bilateral interaction between the cultivars and the Nano-copper fertilizer indicated that the plants of the American cultivar using the Nano-copper fertilizer at a concentration of 1.5 g L^{-1} recorded the highest value in leaf area amounting to 13089.0 cm^{2} Plant^{-1} and significantly outperformed all other treatments of this overlap while the lowest value of leaf area amounted to 7448.2 cm^{2} Plant^{-1} was found in the plants of the American variety in the comparison treatment. The results of the bilateral interaction between the case of pinching and copper Nano-fertilizer in the leaf area indicate that the highest value in this characteristic was found in the case of interaction between the treatment of pinching and the concentration of 1.5 mg L^{-1} of copper amounted to 12283.3 cm^{2} plant^{-1}, while the lowest value amounted to 7855.7 cm^{2} plant^{-1} was found when treated without pinching and without adding copper Nano-fertilizer. The results of the triple interaction between the studied factors (varieties, state of pinching and Nano-copper fertilizer) in the leaf area show that the highest value of this trait was 14204.0 cm^{2}.Plant^{-1} was found in the American cultivar plants and when using pinching and concentration 1.5 g L^{-1} of Nano-copper fertilizer, it excelled this treatment had a significant effect on all the treatments of this interaction, while the lowest value in this trait was 7347.7 cm^{2} plant^{-1}, which was recorded in American cultivar plants without pinching in comparison plants.

Table 4. Effect of cultivars, Case of pinching, Nano-copper fertilizer and the interaction between them on leaf area (cm^{2} plant^{-1}).

Cultivars	**Case of pinching**	**Concentration of Nano- copper fertilizer**			**Cultivars X Case of**	**Average effect of Cultivars**
		0	**1**	**1.5**		
Spanish	Without pinching	7985.7 h-j	9549.0 e-g	10939.7 c-e	9491.4 c	9919.2 a
	pinching	8046.3 g-j	11421.3 b-d	11573.0 b-d	10346.9 ab	
American	Without pinching	7347.7 j	9328.7 f-h	11974.0 bc	9550.1 c	10089.1 a
	pinching	7548.7 ij	10131.7 d-f	14204.0 a	10628.1 a	
Dutch	Without pinching	8233.7 g-j	10442.7 c-f	10527.3 c-f	9734.6 bc	10346.4 a
	pinching	8997.0 f-i	12805.0 b	11073.0 c-e	10958.3 a	
Cultivars X Nano- copper fertilizer	Spanish	8016.0 ef	10485.2 cd	11256.3 bc		
	American	7448.2 f	9730.2 d	13089.0 a	Average effect of pinching case	
	Dutch	8615.3 e	11623.8 b	10800.2 bc		
Case of pinching X Nano- copper fertilizer	Without pinching	7855.7 d	9773.4 c	11147.0 b	9592.0 b	
	pinching	8197.3 d	11452.7 b	12283.3 a	10644.4 a	
Average effect of Nano-copper fertilizer		8026.5 c	10613.1 b	11715.2 a		

Averages that share the same letter for each factor and each interaction do not differ significantly between them according to Duncan's polynomial test at the probability level ($P < 0.05$).

4. Discussion

The differences between the varieties under study in the studied growth characteristics may be explained by the fact that the genetic and phenotypic diversity in the studied traits leads [14], therefore, to the high heritability of those traits increasing most of the growth indicators in the case of using the pinching process, perhaps due to the fact that it results from pinching the growing tip, a decrease in auxin production in the apical bud, an increase in the distribution of nutrients and their growth on the lateral buds, and an increase in branching [15]. ,and significant increases in the traits in which Nano-fertilization was used it may be attributed to the fact that foliar application with copper has an important role in improving plant performance and that its deficiency causes a defect in plant growth through its participation in the photophosphorylation process in photosynthesis and redox processes in the chain transport electron (in aerobic respiration) [16,17].

Conclusions

It is concluded from this study that the use of the process of tip pinching and the use of Nano-copper fertilizer had a significant effect on increasing the number of branches and the leaf area of the plant. Accordingly, we recommend under the conditions of this study to use copper Nano-fertilizer and pinching process when cultivating fields with broad bean.

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Effect of Soaking Tubers in Potassium Humate and Spraying with Nano-Calcium Fertilizer on some Yield Traits of Two Potato Cultivars

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Abstract. In the spring of 2022, researchers from the University of Mosul's Faculty of Agriculture and Forestry conducted an experiment on a vegetable field. A total of three variables were investigated in this experiment (Montreal and EL-Beida). The second variable was a process of soaking tubers in a solution of potassium humate with a concentration of (0, 0.5, 1 g L^{-1}). This experiment used 18 treatments (2 3 3), with the third factor being Nano-calcium fertilizer at three concentrations of (0, 1.5, and 2.5 g L^{-1}) applied to plants at three stages of plant growth: the first 20 days after full germination, the second and third stages, with a 20-day interval between addition and another. Cultivars were put in the main plots and the interaction between two additional variables in sub-plots in a factorial experiment inside a split-plot using the Randomized Complete Block Design with three replicate. The means were compared using the Duncan multiple range test at the 5% significance level for statistical analysis. These findings may be summed up as a whole: In terms of individual plant yield, overall yield, marketing yield of the plant, and marketing yield per unit area, the EL-Beida cultivar was much superior than the Montreal cultivar. There did not seem to be any significant influence of the three parameters on the non-market yield, and the best significant values for individual plant yield and total yield, as well as market yield of the plant and marketing yield per unit area, were all 5 g.L^{-1}.

Keywords. Potato, Yield Traits, Spraying.

1. Introduction

The potato, *Solanum tuberosum* L., belongs to the Solanaceae family, and is considered one of the important vegetable crops rich in nutrients with a large store of energy, which are used in many fields in the food industry, as each 100 g of it contains 79.8 water, 76 calories, and 17.1 carbohydrates. And 2.1 grams of protein, 20 mg of ascorbic acid, 407 mg of potassium, 53 mg of phosphorus, 7 mg of calcium, as well as various other quantities of mineral elements[1,2].Cultivars that bear good characteristics and have high productivity are one of the most important factors that determine the yield [3]. The genetic nature of the cultivated variety also greatly affects the quantity and quality of the yield [4,5]. It is not possible to judge the preference of any potato variety unless They were grown from one rank under similar conditions [6]. Soil pH is maintained and plant nutritional requirements are met during all stages of development thanks to the addition of the fertilizers. It also lessens the need for mineral fertilizers by cutting down on the consumption of nutritional element types[7,8],

Potassium is one of the main nutrients necessary for plant growth, and it is the third most important macronutrient. There are many functions of potassium in plant cells, including vital-physical functions [9], such as increasing the plant's ability to withstand various environmental stresses such as infection with pathogens, exposure to insects, heat tolerance, osmotic regulation, and the movement of carbohydrates from their formation sites to Its storage sites, protein representation and activation of enzymes, as it activates more than 70 enzymes such as oxidation and reduction enzymes and other enzymes and increases the efficiency of the photosynthesis process as well as other important functions of the plant, including the representation of carbohydrates and the regulation of the mechanism of opening and closing stomata [10]. Potassium is involved in increasing the process of photosynthesis, and its deficiency leads to the destruction of chloroplasts. It is also important in building energy, and it is the main carrier of energy in the plant, and its deficiency is reflected in the process of photosynthesis [11]. Organic acids play an important role as stimulants for the potato plant, as it accelerates ripening and neglects to increase the yield and improve the quality. In addition, it increases the plant's ability to tolerate diseases[12,13]. Calcium is one of the essential nutrients for plant growth in addition to being essential in building plant cell walls. In addition, it plays an important role in increasing plant tolerance to salt stress conditions, which negatively affect plant growth and production [14,15] where calcium reduces sodium absorption.

Research aims:

Studying the yield of two cultivars of potatoes and showing the effect of soaking the tubers before planting with the humic compound (Nerohum) and knowing the importance of using spraying with calcium Nano-fertilizer and finding the best interactions between cultivars and concentrations of soaking the tubers with organic compounds and spraying with Nano-calcium fertilizer on yield characteristics and its components in potatoes.

2. Materials and Methods

The study was conducted at Mosul University during the 2022 spring growing season on the college's research area. The field was ploughed to get it ready for planting. A 2.10 m long and 75 cm wide set of ridges made up the experimental unit. Hence, after that expansion, the experimental unit covered a total of 7.875 square meters. Seven tubers were planted in each seedbed at a spacing of 30 centimeters apart (35 plants per experimental unit). Surface irrigation was used to water the plants in the field after the tubers were planted at a depth of 12-15 cm. All experimental units received standard agricultural services such fertilization, weeding, export, and pest, disease, and shrub control. Urea (46% N), triple superphosphate (45% P_2O), and potassium sulfate (48% K_2O) were all applied at rates of 400 kg per hectare. All of the phosphate fertilizer was added 15 days after planting, half of the nitrogen fertilizer and all of the potassium fertilizer were added after emergence, and the remaining half of the nitrogen fertilizer was added one month after the second application, also in a trench directly under the plant. First, two exotic potato varieties were used in the experiments (Montreal and EL-Beida), second, the tubers were soaked in a solution of potassium humate (at a concentration of (0, 0.5, 1 g L^{-1}). Nano-calcium fertilizer, available in three different strengths, rounded out the list (0, 1.5 and 2.5 g L^{-1}). Plants given Nano-calcium fertilizer at three different times: The trial included 18 treatments (2 3 3), each lasting 20 days and occurring either immediately after the previous treatment or 20 days afterwards. Cultivars were organized in the main plots and the interaction between two additional variables in the sub-plots, and each treatment was replicated three times in a factorial experiment inside a split-plot using the Randomized Complete Block Design with three replicate. The [16]. software was used to do the statistical analysis. Individual plant yield, marketing yield, total tuber yield per unit area, marketing yield per unit area, and non-marketing yield per unit area were all measured.

3. Results and Discussion

3.1. Yield (g. Plant^{-1})

Table (1) shows that the employed cultivars had an effect on the trait of plant yield, and that the EL-Beida cultivar was much better than the Montreal cultivar in this attribute. Spraying with calcium

Nano-fertilizer at a concentration of 1.5 g.L^{-1} produced the highest value, followed by soaking with potassium humate at 1 g L^{-1} (with increases of 6.57 and 7.83%, respectively, compared to the concentration of 0.5 g.L^{-1} and the control treatment) for the best overall significant value in this characteristic.

The results of the binary interaction between cultivars and soaking with potassium humate indicate that the cultivar EL-Beida in the case of soaking with potassium humate at a concentration of 1 g.L^{-1} recorded the highest significant value in this trait amounted to 1133.89 g.Plant-1, and it differed significantly only with the interaction of the Montreal cultivar with all concentrations of soaking used ,while the lowest value in this trait was recorded from the interaction treatment of the Montreal variety when soaking with potassium humate was not used, as the yield per plant was 921.78 g. $Plant^{-1}$. Cultivar EL-Beida in the case of interaction with the treatment of spraying with calcium Nano-fertilizer at a concentration of 1.5 g.L^{-1} gave the best significant value in this trait amounted to 1157.67 g.$Plant^{-1}$, significantly superior in this value to the plants of the same cultivar not treated with spraying with calcium Nano-fertilizer and on all the interaction treatments in the case of the cultivar EL-Beida, the lowest value in this trait was recorded from the interaction treatment of the Montreal cultivar when not using calcium Nano-fertilizer, which amounted to 921.33 g.$Plant^{-1}$. The highest significant value in this attribute, 1194.00 g, was observed for the binary interaction treatment of soaking with potassium humate at a dosage of 1 g.L^{-1} and spraying with calcium Nano-fertilizer at a concentration of 1.5 g.L^{-1}. Except for the interaction treatment in the case of not soaking with potassium humate and spraying with calcium Nano-fertilizer at a dosage of 2.5 g, Plant-1 is considerably better than all other treatments for this attribute. L^{-1}.

Table (1) Effect of cultivars, soaking tubers with potassium humate and spraying with Nano- calcium fertilizer on the yield of the individual plant (g. $Plant^{-1}$).

Cultivars	**Soaking Con. (g .L^{-1})**	**Nano- calcium Con. (g.L^{-1})**			**Cultivar × Soaking**	**Average effect of cultivar**
		0	**1.5**	**2.5**		
EL-Beida	0	943.67 d-f	1072.00 b-e	1185.67 ab	1067.11 ab	1085.56 a
	0.5	999.33 c-f	1126.67 a-c	1041.00 b-e	1065.67 ab	
	1	1088.67 b-e	1274.33 a	1038.67 b-e	1133.89 a	
Montreal	0	850.67 f	929.00 ef	985.67 c-f	921.78 d	963.07 b
	0.5	970.33 c-f	936.00 d-f	964.00 c-f	965.78 cd	
	1	943.00 d-f	1113.67 a-d	975.33 c-f	1010.67 bc	
Cultivar × Nano-calcium	EL-Beida	1010.56 bc	1157.67 a	1088.44 ab	Average effect of Soaking	
	Montreal	921.33 c	992.89 c	975.00 c		
Soaking × Nano-calcium	0	897.17 c	1000.50 bc	1085.67 ab	994.44 b	
	0.5	984.83 bc	1031.33 b	1002.50 bc	1006.22 b	
	1	1015.83 bc	1194.00 a	1007.00 bc	1072.28 a	
Average effect of Nano-calcium		965.94 b	1075.28 a	1031.72 a		

* The averages that share the same alphabetic letter for each factor and each interference do not differ significantly among themselves according to Duncan's polynomial test at the probability level ($P \leq 0.05$).

3.2. Marketing Yield of the Plant (g. Plant^{-1})

Marketing yield was significantly higher in the EL-Beida cultivar than in the Montreal cultivar (Table 2). Soaking with potassium humate at both concentrations resulted in a positive increase that did not reach the significant level in this trait, and spraying with calcium Nano-fertilizer resulted in a significant increase in the marketing yield of 9.76% at a concentration of 1.5 g.L^{-1}. As for the results of the binary interaction between cultivars and soaking with potassium humate, it was found that the interaction treatment that included the cultivar EL-Beida and soaking with potassium humate at a concentration of 1 g.L^{-1} gave the highest significant value for this trait amounted to 1044.22 g plant^{-1}, and the interaction treatment that included the use of the Montreal cultivar and not using soaking with potassium humate, gave the lowest value for this trait amounting to 847.11 g plant^{-1}. The results of the bilateral interaction between cultivars and spraying with calcium Nano-fertilizer showed the superiority of the interaction treatment of the EL-Beida variety with spraying calcium Nano-fertilizer at a concentration of 1.5 g.L^{-1}, as the marketing yield in this treatment reached 1081.56 g plant-^{1}, superior to all other interaction treatments except for the interaction treatment of the same cultivar in the case of spraying with calcium Nano-fertilizer at a concentration of 2.5 g.L^{-1}, and the lowest value for this characteristic amounted to 873.89 g.plant^{-1} recorded in the interaction treatment of the Montreal variety and when not sprayed with calcium Nano-fertilizer. The highest significant value in this characteristic was 1104.33 g.plant^{-1}, and it was found in the treatment of interaction between soaking with potassium humate at a concentration of 1g.L^{-1} and spraying with calcium Nano-fertilizer at a concentration of 1.5 g.L^{-1}, significantly different from all other interaction treatments. The lowest value for this characteristic was found in the treatment of interaction that included not soaking with potassium humate. The highest marketing yield for the plant was 1178.00 g.plant^{-1} when the EL-Beida cultivar was combined with soaking at a concentration of 1 g.L^{-1} of potassium humate and spraying with calcium Nano-fertilizer at a concentration of 1.5 g L^{-1}. This was a significant improvement over the control treatment.

Table 2. Effect of cultivars, soaking tubers with potassium humate and spraying with Nano- calcium fertilizer on the marketing yield of the plant (g. Plant^{-1}).

Cultivars	**Soaking Con. (g .L^{-1})**	**Nano- calcium Con. (g.L^{-1})**			**Cultivar × Soaking**	**Average effect of cultivar**
		0	**1.5**	**2.5**		
EL-Beida	0	872.67 c-e	1004.00 a-e	1111.67 ab	996.11 a	1016.48 a
	0.5	968.00 b-e	1062.67 a-c	996.67 a-e	1009.00 a	
	1	1004.33 a-e	1178.00 a	950.33 b-e	1044.22 a	
Montreal	0	808.33 e	883.00 c-e	850.00 de	847.11 b	890.96 b
	0.5	921.33 b-e	841.67 de	895.00 c-e	886.00 b	
	1	892.00 c-e	1030.67 a-d	896.67 c-e	939.78 ab	
Cultivar × Nano-calcium	EL-Beida	948.33 bc	1081.56 a	1019.56 ab	Average effect of Soaking	
	Montreal	873.89 c	918.44 bc	880.56 c		
Soaking × Nano-calcium	0	840.50 c	943.50 bc	980.83 b	921.61 a	
	0.5	944.67 bc	952.17 bc	945.83 bc	947.56 a	
	1	948.17 bc	1104.33 a	923.50 bc	992.00 a	
Average effect of Nano-calcium		911.11 b	1000.00 a	950.06 ab		

* The averages that share the same alphabetic letter for each factor and each interference do not differ significantly among themselves according to Duncan's polynomial test at the probability level ($P \leq 0.05$).

3.3. Total Yield (ton ha^{-1})

Table 3 shows that the cultivars chosen had a substantial impact on overall output, with EL-Beida producing the greatest yields (+12.72%) and Montreal producing the lowest. When comparing the effects of soaking with potassium humate at concentrations of 0.5 and 0 g L^{-1}, the total yield was found to rise by 6.57 and 7.83%, respectively, when treated with potassium humate at a concentration of 1 g L^{-1}. The greatest statistically significant increase in overall yield was seen when calcium nano-fertilizer was sprayed at a concentration of 1.5 g.L^{-1}. It reached 47.790 tons ha^{-1} and was different from the control treatment where calcium Nano-fertilizer was not sprayed, but was not noticeably different from the treatment in which this fertilizer was sprayed at a concentration of 2.5 g.L^{-1}. Results from a binary interaction analysis between cultivars and potassium humate soaking showed that the combination of the EL-Beida cultivar and soaking with potassium humate at a concentration of 1g.L^{-1} produced the highest total yield of 50.395 ton.ha-1, surpassing only the interaction treatments of the Montreal variety with all soaking concentrations. When the overlap of the EL-Beida cultivar was treated with a spray of calcium Nano-fertilizer at a concentration of 1.5 g.L^{-1}, total yield increased significantly to 51.452 tons.ha-1, outperforming all other treatments of this interaction with the exception of the treatment of the interaction of the same variety. The interaction treatment that included soaking with potassium humate at a concentration of 1 g.L^{-1} and spraying with calcium Nano-fertilizer at a concentration of 1.5 g.L^{-1} resulted in the highest significant value in total yield, 53.067 ton.ha^{-1}, compared to all other interaction treatments except the overlap treatment. that did not involve soaking with potassium humate and spraying with calcium Nano-fertilizer at a concentration of 2.5 g.L^{-1}, with the lowest value of total yield coming from the interaction treatment in the case of not soaking with potassium humate and not spraying with calcium Nano-fertilizer (39.874 ton.ha^{-1}). The highest significant value in total yield reached 56.637 tons.ha^{-1} in the treatment of the interaction of cultivar EL-Beida, soaking at a concentration of 1 g.L^{-1},and spraying with calcium Nano-fertilizer at a concentration of 1.5 g.ha^{-1}. These results are from the study's three-way interaction between the three factors of interest (cultivars, soaking with potassium humate, and spraying with calcium Nano-fertilizer).

3.4. Marketing Yield (ton ha^{-1})

According to Table (4), the EL-Beida variety outperformed the Montreal variety by a wide margin. The EL-Beida variety provided a marketing yield of 45.177 tons.ha^{-1}, a growth rate of 14.089% over the Montreal variety's 39.598 tons.ha-1. When compared to the control treatment, the marketing yield varied significantly across the two potassium humate soaking concentrations (0.5 and 1 g.L^{-1}), with the highest significant value in this characteristic being 44.444 ton.ha^{-1}, achieved through the application of calcium Nano-fertilizer at a concentration of 1.5 g.L^{-1}. Only the 0 g.L-1 concentration treatment with calcium Nano-fertilizer showed a substantially different result for this feature (40.494 ton.ha^{-1}). Marketing yield reached 40.410 tons. ha^{-1}in the treatment of the binary interaction between the cultivar EL-Beida and the soaking with potassium humate at a concentration of 1 g.L^{-1}, and the treatment of this interaction was significantly superior only to the comparison and concentration treatments 0.5 g. L^{-1}in the case of Montreal cultivar, while the value of the market yield was highest in the treatment of the interaction between the cultivar EL-Beida and the soaking with potassium All treatments of this interaction except for the interaction treatment for the same cultivar when spraying at a concentration of 2.5 g L^{-1} of calcium Nano-fertilizer resulted in significantly lower marketing yields than the treatment involving the bilateral interaction between the cultivar EL-Beida and spraying with a concentration of 1.5 g. L^{-1} of calcium Nano-fertilizer, which gave the highest significant value of the marketing yield at 48.069 tons.ha^{-1}. According to the results of the dual interaction between soaking with potassium humate and spraying with calcium Nano-fertilizer, the treatment that used soaking with potassium humate at a concentration of 1 g.L^{-1} and spraying with calcium Nano-fertilizer at a concentration of 1.5 g.L^{-1} gave the highest significant value of the marketing yield, which amounted to 49.081 ton.ha^{-1}. According to the summary of the results of the triple interaction between the three

factors (cultivars, soaking with potassium humate, and spraying with calcium Nano-fertilizer), the highest significant value in this trait was 52.356 tons.ha-1, and this was achieved in the treatment of the interaction of cultivar EL-Beida and soaking with potassium humate at a concentration of 1 $g.L^{-1}$ and spraying with a concentration of 1.5 $g.L^{-1}$ of calcium.

Table 3. Effect of cultivars, soaking tubers with potassium humate and spraying with Nano- calcium fertilizer on the total yield of tubers per unit area (ton. ha^{-1}).

Cultivars	Soaking Con. ($g.L^{-1}$)	Nano- calcium Con. ($g.L^{-1}$)			Cultivar × Soaking	Average effect of cultivar
		0	1.5	2.5		
EL-Beida	0	41.941 d-f	47.644 b-e	52.696 ab	47.427 ab	48.247 a
	0.5	44.415 c-f	50.074 a-c	46.267 b-e	46.919 ab	
	1	48.385 b-e	56.637 a	46.163 b-e	50.395 a	
Montreal	0	37.807 f	41.289 ef	43.807 c-f	40.968 d	42.803 b
	0.5	43.126 c-f	41.600 d-f	42.844 c-f	42.523 cd	
	1	41.911 d-f	49.496 a-d	43.348 c-f	44.919 bc	
Cultivar × Nano-calcium	EL-Beida	44.914 bc	51.452 a	48.375 ab	Average effect of Soaking	
	Montreal	40.948 c	44.128 c	43.333 c		
Soaking × Nano-calcium	0	39.874 c	44.467 bc	48.252 ab	44.198 b	
	0.5	43.770 bc	45.837 b	44.556 bc	44.721 b	
	1	45.148 bc	53.067 a	44.756 bc	47.657 a	
Average effect of Potassium		42.931 b	47.790 a	45.854 a		

* The averages that share the same alphabetic letter for each factor and each interference do not differ significantly among themselves according to Duncan's polynomial test at the probability level ($P \leq 0.05$).

Table 4. Effect of cultivars, soaking tubers with potassium humate and spraying with Nano- calcium fertilizer on the marketing yield of tubers per unit area (ton. ha^{-1}).

Cultivars	Soaking Con. ($g.L^{-1}$)	Nano- calcium Con. ($g.L^{-1}$)			Cultivar × Soaking	Average effect of cultivar
		0	1.5	2.5		
EL-Beida	0	38.785 c-e	44.622 a-e	49.407 ab	44.272 a	45.177 a
	0.5	43.022 b-e	47.230 a-c	44.296 a-e	44.849 a	
	1	44.637 a-e	52.356 a	42.237 b-e	46.410 a	
Montreal	0	35.926 e	39.244 c-e	37.778 de	37.649 b	39.598 b
	0.5	40.948 b-e	37.407 de	39.778 c-e	39.378 b	
	1	39.644 c-e	45.807 a-d	39.852 c-e	41.768 ab	
Cultivar ×	EL-Beida	42.148 bc	48.069 a	45.314 ab	Average effect of	

Cultivars	Soaking Con. (g .L^{-1})	Nano- calcium Con. (g.L^{-1})			Cultivar × Soaking	Average effect of cultivar
		0	1.5	2.5		
Nano- calcium	Montreal	38.840 c	40.820 bc	39.136 c		
Soaking × Nano- calcium	0	37.356 c	41.933 bc	43.593 b	40.960 a	
	0.5	41.985 bc	42.319 bc	42.037 bc	42.114 a	
	1	42.141 bc	49.081 a	41.044 bc	44.089 a	
Average effect of Nano- calcium		40.494 b	44.444 a	42.225 ab		

* The averages that share the same alphabetic letter for each factor and each interference do not differ significantly among themselves according to Duncan's polynomial test at the probability level (P≤ 0.05).

3.5. Non- Marketing Yield of Tubers Per Unit Area (ton. ha^{-1})

Table (5) shows that there was no statistically significant difference in non-market yield between the two studied cultivars (EL-Beida and Montreal), nor between the two soaking treatments with potassium humate (1.5 and 2.5 g.L^{-1}), nor between the control treatment and the application of calcium Nano-fertilizer (1.5 and 2.5 g.L^{-1}). The lowest value of the non-market yield was achieved with the EL-Beida cultivar when soaking with potassium humate at a concentration of 0.5 g.L^{-1}, according to the results of the binary interaction between cultivars and soaking with potassium humate. Other than the interaction treatment of the same cultivar when soaking with potassium humate at a concentration of 1.5 g.L^{-1}, this treatment did not substantially vary from the other treatments of this interaction.

the value of the non-marketing yield was highest in the treatment of the Montrea variety when soaked with a concentration of 5.0 g.L^{-1} of potassium humate and when not sprayed with calcium Nano-fertilizer, it was lowest in the treatment of the interaction of the EL-Beida variety when soaking with a concentration of 0.5 g.L^{-1} of potassium humate and when not sprayed with calcium Nano-fertilizer, where it was recorded at ha^{-1}.

Table 5. Effect of cultivars, soaking tubers with potassium humate and spraying with Nano- calcium fertilizer on the non- marketing yield of tubers per unit area (ton. ha^{-1}).

Cultivars	Soaking Con. (g .L^{-1})	Nano- calcium Con. (g.L^{-1})			Cultivar × Soaking	Average effect of cultivar
		0	1.5	2.5		
EL-Beida	0	3.156 ab	3.022 ab	3.289 ab	3.156 ab	3.070 a
	0.5	1.393 b	2.844 ab	1.970 b	2.069 b	
	1	3.748 ab	4.281 ab	3.926 ab	3.985 a	
Montreal	0	1.881 b	2.044 b	6.030 a	3.319 ab	3.205 a
	0.5	2.178 b	4.193 ab	3.067 ab	3.146 ab	
	1	2.267 b	3.689 ab	3.496 ab	3.151 ab	
Cultivar × Nano- calcium	EL-Beida	2.765 ab	3.383 ab	3.062 ab	Average effect of Soaking	
	Montreal	2.109 b	3.309 ab	4.198 a		
Soaking × Nano- calcium	0	2.519 ab	2.533 ab	4.659 a	3.237 a	
	0.5	1.785 b	3.519 ab	2.519 ab	2.604 a	

Cultivars	Soaking Con. (g .L^{-1})	Nano- calcium Con. (g.L^{-1})			Cultivar × Soaking	Average effect of cultivar
		0	1.5	2.5		
	1	3.007 ab	3.985 ab	3.711 ab	3.568 a	
Average effect of Nano-calcium		2.437 a	3.346 a	3.630 a		

* The averages that share the same alphabetic letter for each factor and each interference do not differ significantly among themselves according to Duncan's polynomial test at the probability level ($P \leq 0.05$).

That is consistent with what was said in [17] that the variations in yield characteristics of the cultivars under investigation may be related to the various genotypes of the two cultivars tested. Potassium's role in activating many enzymes that speed up the transfer of carbohydrates from manufacturing places to storage places may account for the significant increase in plant yield and total yield as a result of soaking potato tubers with potassium humate before planting, compared to not soaking. Considering his remarks [18]. Calcium's effect on yield and its constituents is evident in the fact that this element helps boost the performance of photosynthesis, which in turn boosts plant development and, ultimately, yield [19, 20]. That is also consistent with what [21,22,23] say that the element calcium plays a role in regulating physiological processes that boost function to improve production in potatoes.

Conclusions

As compared to Montreal cultivars, the EL-Beida variety performed better across the board. This research suggests that the EL-Beida cultivar be used in Nineveh Governorate because of the superior yield it provides. Plant production and overall yield were significantly increased after soaking with potassium humate at a concentration of 1g.L-1. The research suggests using humate molecules at varying doses to accomplish the desired yield enhancement. Research showed that using calcium Nanoparticles in foliar spray at doses of 1.5 and 2.5 gm.L-1 significantly increased most yield parameters.

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The Effect of Adding Zeolite and Foliar Application of Nano Potassium on Growth of Radish *Raphanus sativus* L.

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Abstract. The experiment was carried out in the field of the College of Agriculture, University of Kerbala, in a mixed sandy soil during the winter agricultural season for the year 2022-2023, to study the effect of adding zeolite with three concentrations (0, 10, 20 t ha^{-1}) and spraying with potassium nanoparticles with three concentrations also (0, 1, 2 gm L^{-1}) on some growth characteristics of two types of radish, Spanish black radish and local red. The study was carried out as a factorial experiment with a randomized complete block design (RCBD) with three replications. The results showed a significant effect of the single factors of zeolite and nano-potassium on vegetative traits (plant height, number of leaves, leaf area, chlorophyll content) and carbohydrates for both types, but the results indicated the response of black radish in the double and triple interactions of the studied factors. The triple overlap treatment (10 t ha^{-1} zeolite + 0 gm L^{-1} potassium for black radish) recorded the highest values in the number of leaves and leaf area 11.53 leaf-1, 5400 cm^2 respectively, while the treatment (10 t ha^{-1} zeolite + 1 gm L^{-1} for black radish) excelled and gave the highest percentage of carbohydrates, reaching 11.61%. It can be concluded from the study to the clear vital role played by natural zeolite, even with few additions, in improving the qualitative and quantitative growth characteristics of various agricultural crops, and it can be relied upon in sustainable agriculture.

Keywords. Black radish, Red radish, Zeolite, Sustainable Agriculture, Spanish radish.

1. Introduction

The radish *Raphanus sativus* L. is one of the winter vegetable crops belonging to the Brassicaceae family. It is a vegetable that has been used since ancient times. It grows in a moderate climate and does not tolerate high temperatures, but it is resistant to low temperatures. Its origin dates back to central and western China and India[1]. Radish is an edible root plant with a height of between 30-90 cm. Its roots are thick and of a variety of sizes, shapes and colors [2]. Radish is grown for its leaves and roots, which are eaten fresh and cooked, and drunk as juice because of its benefits. Radish contains sugars, dietary fibers, carbohydrates, proteins, and vitamins such as vitamin A, vitamin B (B1, B2, B3, B5, B6, B9), ascorbic acid, and minerals such as calcium. potassium, phosphorus, iron, magnesium, sodium, zinc, and fluoride, in addition to flavonoids, alkaloids, tannins, and phenolic compounds, black radish contains glycosides (glucosinolates and isothiocyanates) phytochemicals [3,4].

In recent years, the trend towards sustainable agriculture has become the focus of attention of researchers through the use of various means, including the use of natural minerals as soil conditioners that work to improve the physical and chemical properties of soil and increase its water retention .
Biostimulants are substances used on seeds, plants, and soil that can be either natural or synthetic [5]. Changes in basic and structural functions are induced by these compounds, which then affect plant growth by making plants more resistant to abiotic stress and boosting the yield and quality of seeds and grains. Biostimulants also reduce the need for fertilizers [6].
Zeolite is one of the minerals that have these potentials and is of great interest in the agricultural field to improve soil properties [7]. Zeolite is a natural and synthetic mineral formed by the alteration of glass-rich volcanic rocks when they interact with sea water [8]. This mineral works to improve the physical properties of the soil by increasing the porosity and has the characteristic of ion exchange with positive ions, and its fragile structural structure contributes to soil aeration [9].
Several technologies have recently been introduced in science, including agricultural sciences, to improve production in quantity and quality. Nanotechnology is one of these technologies that have been used to improve and develop chemical fertilizers and pesticides [10,11]. Compounds whose particles range from 1-100 nanometers are called nanomaterials, as these particles are characterized by their small size and large surface area to be ideal in the manufacture of fertilizers, which are called smart fertilizers after being encapsulated or made chelated, so they become slow to release and suit the stages of plant growth [12-14]. The possibility of using nano-fertilizer technology to reduce the excessive use of traditional chemical fertilizers in agricultural production and reduce its impact on the environment by relying on low concentrations of nano-fertilizers and reaching the desired results from them, and this is what studies have indicated over the past years to the important and vital role that potassium element plays in Improving the quantitative and qualitative traits of various field and horticultural crops [15-17].
Based on the foregoing, this study aims to use natural and locally produced zeolite as a soil conditioner and its effect on the vegetative growth of two types of radishes in interaction with the use of potassium nanoparticles. It also aims to test the black radish (the Spanish variety) under the conditions of the study area and its response to the study factors compared with the common local variety.

2. Materials and Methods

For the 2022-2023 agricultural winter season, the experiment was carried out on a field at the University of Karbala's College of Agriculture. The research involved three variables: application of natural zeolite at three different rates (0, 10, and 20 t ha^{-1}), nano-potassium at three different rates (0, 1, and 2 gm L^{-1}), and two different varieties of radish (Spanish black and local red). As a result, in a factorial experiment employing the randomized complete block design (R.C.B.D.) with three replications, the number of overlapping treatments is 18. Plants were spaced 20 centimeters apart in lines and 15 centimeters apart in rows over the whole experimental field, for a total of 54 experimental units, each with an area of 1 square meter.
Both varieties of radish seeds were planted immediately in the field, and the experimental plots were watered using drip irrigation. When the field was ploughed, levelled, and divided into the experimental units, zeolite was added immediately. The first application of potassium fertilizer was made when the plant had four true leaves, and a second application was made two weeks later. Plant height (cm), number of leaves (leaf. $plant^{-1}$), leaf area (cm^2. $plant^{-1}$) according to the method of [18], chlorophyll content in leaves ($mg.gm^{-1}$ fresh weight) according to the method of [19], and the percentage of total carbohydrates in leaves (%) were measured after the crop matured (roughly 60-70 days after planting). Data was analysed statistically with Genstat, and means were compared using the least-significant-difference test at the 0.05 significance level.

3. Results and Discussion

3.1. Plant Height (cm)

Table (1) shows that the zeolite treatments were better in plant height (cm) to the Z2 level, which had the greatest average height of 30.87 cm, Z1, which had 30.47 cm, and the comparison treatment, which had 25.60 cm. The same table demonstrates that the spraying treatments with nano-potassium differed in plant height, with K2 having the greatest average of 30.87 cm and K1 and K0 having the lowest averages of 27.98 cm and 28.22 cm, respectively. The black radish had the largest average plant height of 39.27 cm, compared to 18.68 cm for the red radish. As shown in the table, the bilateral interaction between zeolite treatments and type had a significant effect on plant height. Treatment BZ2 had the highest average plant height of 43.20 cm, followed by BZ1 at 41.44 cm and RZ0 at 18.01 cm. The table showed that BK2 had the greatest average plant height of 42.51 cm compared to RK0, which had the lowest value of 17.87 cm. It generated the largest plant height, 32.19 cm, compared to the comparator treatment's 22.65 cm (Table 1). The same table reveals that BZ2K2, BZ1K0, and BZ2K1 had the highest average plant heights of 46.55, 45.70, and 42.72 cm, respectively, after adding zeolite and spraying nano-potassium on the two varieties of radish. height, which amounted to 14.47 cm.

Table 1. Effect of adding zeolite and spraying with nano-potassium and their interactions on the plant height (cm) of two types of radish.

Radish type (T)	Zeolite (Z) t ha^{-1}	Nano-Potassium (K) gm L^{-1}			Interaction Z × T	T Mean
		0 (K0)	1(K1)	2 (K2)		
Black (B)	0 (Z0)	29.63	28.40	41.50	33.18	39.27
	10 (Z1)	45.70	39.07	39.55	41.44	
	20 (Z2)	40.41	42.72	46.55	43.20	
Red (R)	0 (Z0)	14.47	20.51	19.05	18.01	18.68
	10 (Z1)	20.31	18.27	19.89	19.49	
	20 (Z2)	18.82	18.91	17.91	18.55	
K Mean		28.22	27.98	30.87	L.S.D 0.05	L.S.D 0.05
L.S.D 0.05T × Z × K			4.45		Z × T 2.56	T 1.98
Interaction T × K	Black	38.58	36.73	45.51	L.S.D 0.05T × K 2.56	
	Red	17.87	19.23	18.95	Z mean	
Interaction Z × K	0 (Z0)	22.65	24.46	30.28	25.60	
	10 (Z1)	33.00	28.67	29.72	30.47	
	20 (Z2)	29.61	30.82	32.19	30.87	
		L.S.D 0.05				
Z × K		K		Z		
3.14		1.81		1.81		

3.2. Number of Leaves (leaf plant^{-1})

Table (2) demonstrates that zeolite treatments had the largest average number of leaves, with treatment Z1 having 9.14 leaf plant^{-1} and treatment Z2 having 9.00 leaf plant^{-1}. The comparison treatment had 7.69 leaf plant^{-1}. The same table shows that nano-potassium treatments did not affect the characteristic. The red radish had the lowest average number of leaves, whereas the black radish had 10.50 leaf plant^{-1}. 6.89 leaf-plant^{-1}. Treatment BZ1 had the largest average number of leaves, 11.35, 11.08 leaf plant^{-1}, compared to treatment RZ0, which had the lowest average of 6.84 leaf plant^{-1}. The same data indicates the advantage of nano-potassium treatments with the type of radish, as BK2 had the greatest average number of leaves, 10.68 leaf plant^{-1}, followed by BK0, 10.44 leaf plant^{-1}, and BK1, 10.38 leaf plant^{-1}, compared to RK1, 6.73 leaf plant^{-1}. From the bilateral overlap between zeolite and nano-potassium treatments, the Z1K2 treatment is superior, giving the highest average number of leaves of 9.63 leaf plant^{-1}, which did not differ from the Z1K0 treatment, which recorded 9.26 leaf plant^{-1}, and the comparison treatment, which recorded 7.65 leaf plant^{-1}.

Table 2. Effect of adding zeolite and spraying with nano-potassium and their interactions on the number of leaves (leaf. plant^{-1}) of two types of radish.

Radish type (T)	Zeolite (Z) t ha^{-1}	Nano-Potassium (K) gm L^{-1}			Interaction Z × T	T Mean
		0 (K0)	1(K1)	2 (K2)		
Black (B)	0 (Z0)	8.63	8.73	9.86	9.07	10.50
	10 (Z1)	11.53	11.33	11.20	11.35	
	20 (Z2)	11.16	11.10	11.00	11.08	
Red (R)	0 (Z0)	6.66	7.00	6.86	6.84	6.89
	10 (Z1)	7.00	6.93	6.86	6.93	
	20 (Z2)	7.20	6.26	7.26	6.91	
K Mean		8.70	8.56	8.84	L.S.D 0.05	L.S.D 0.05
L.S.D 0.05T × Z × K			0.9816		Z × T 0.5667	T 0.3272
Interaction T × K	Black	10.44	1038	10.68	L.S.D 0.05T × K 2.569	
	Red	6.95	6.73	7.00	Z mean	
Interaction Z × K	0 (Z0)	7.65	7.86	8.36	7.69	
	10 (Z1)	9.26	9.13	9.63	9.14	
	20 (Z2)	9.18	8.68	9.13	9.00	
L.S.D 0.05						
Z × K			K		Z	
0.6941			0.4007		0.4007	

It is noted from the triple overlap between the studied factors that there are significant differences in the superiority of the BZ1K0 treatment, which recorded the highest average number of leaves, which amounted to 11.53 leaf plant^{-1}, which did not differ significantly from the BZ1K1 treatment, which gave 11.33 leaf plant^{-1} compared to the comparison treatment on red radish, which recorded the lowest average measured 6.66 leaf plant^{-1}.

3.3. Leaf Area (cm^2 plant^{-1})

The statistical analysis revealed substantial variations in leaf area as a result of the zeolite treatments, with the Z1 treatment performing best and yielding the highest average leaf area of 2969 cm^2 plant^{-1}; this value did not differ significantly from that of the Z2 treatment, which produced 2836 cm^2 plant^{-1}; nor did it differ significantly from that of the comparison treatment, which produced the lowest average of 2163 cm^2 plant^{-1}. The application of nano-potassium had no discernible influence on this attribute, and the black radish plant was clearly better in terms of leaf area, with a mean of 3781 cm^2 plant^{-1} compared to the red radish crop's 1530 cm^2 plant^{-1} (Table 3). The same data shows that the two-way interaction between zeolite treatments and radish types has a considerable effect, with treatment BZ1 producing the largest average leaf area (4565 cm^2 plant^{-1}) and treatment RZ0 producing the lowest (1345 cm^2 plant^{-1}).

The table also shows the bilateral interaction between nano-potassium and the type of radish, as treatment BK0 recorded the highest average leaf area of 3836 cm^2 plant^{-1}, which did not differ significantly from the two treatments BK2 and BK1, which gave 3789, 3718 cm^2 plant^{-1}, respectively, compared to treatment RK0, which recorded the lowest average area It amounted to 1495 cm^2 plant^{-1}, while the same table shows that there are significant differences for the bilateral interaction between zeolite and nano-potassium, where the Z1K0 treatment excelled by giving it the highest average leaf area, reaching 3387 cm^2 plant^{-1}, which did not differ from the Z2K1 treatment, which recorded 3096 cm^2 plant^{-1} compared to With the comparison treatment that gave the lowest average of 1831 cm^2 plant^{-1}. It is clear from Table (3) that the three-way interaction of the study treatments had a significant effect, as the treatments BZ1K0 and BZ2K1 recorded the highest averages of 5400, 4331 cm^2 plant^{-1}, respectively, while the treatment RZ0K0 gave the lowest average for this trait amounted to 1125 cm^2 plant^{-1}.

Table 3. Effect of adding zeolite and spraying with nano-potassium and their interactions on the leaf area (cm^2 $plant^{-1}$) of two types of radish.

Radish type (T)	Zeolite (Z) t ha^{-1}	Nano-Potassium (K) gm L^{-1}			Interaction	T Mean
		0 (K0)	1(K1)	2 (K2)	Z × T	
Black (B)	0 (Z0)	2537.0	2566.0	3839.0	2980.0	3781.0
	10 (Z1)	5400.0	4251.0	4053.0	4565.0	
	20 (Z2)	3572.0	4337.0	3486.0	3798.0	
Red (R)	0 (Z0)	1125.0	1531.0	1381.0	1345.0	1530.0
	10 (Z1)	1375.0	1291.0	1453.0	1373.0	
	20 (Z2)	1984.0	1854.0	1780.0	1873.0	
K Mean		2665.0	2638.0	2664.0	L.S.D 0.05	L.S.D 0.05
L.S.D 0.05T × Z × K			1400.6		Z × T 808.7	T 466.9
Interaction T × K	Black	3836.0	3718.0	3789.0	L.S.D 0.05T × K 808.7	
	Red	1495.0	1558.0	1538.0	Z mean	
Interaction Z × K	0 (Z0)	1831.0	2048.0	3610.0	2163.0	
	10 (Z1)	3387.0	2771.0	2748.0	2969.0	
	20 (Z2)	2778.0	3096.0	2633.0	2836.0	
L.S.D 0.05						
Z × K			K		Z	
990.4			571.8		571.8	

3.4. Leaves Chlorophyll Content (mg gm^{-1} fresh weight)

Treatment Z1 had the highest chlorophyll content of 1.688 mg gm^{-1}, which did not differ significantly from treatment Z2's 1.654 mg gm^{-1}, and both treatments surpassed the comparison treatment's 1.645 mg gm-1 average. In contrast, there was no effect of the individual treatments of nano-potassium on the chlorophyll content, and the black radish excelled with its chlorophyll content. Due to a substantial influence of the bilateral interaction between the zeolite treatments and the kind of radish, the BZ1 treatment performed best and produced the greatest average of chlorophyll, at 1.702 mg gm^{-1}, while the RZ0 treatment performed worst, at 1.626 mg gm^{-1}. From the same table, it can be seen that there are substantial differences in the binary interaction between nano-potassium and the type of radish, with the treatment BK2 recording the highest value of 1.689 mg gm^{-1} and the treatment RK0 giving the lowest mean for chlorophyll, amounting to 1.632 mg gm^{-1}. The table also demonstrates that Z1K2 treatment, which recorded the greatest chlorophyll content of 1.710 mg gm^{-1}, is superior to the control treatment, which recorded the lowest average of 1.625 mg gm^{-1} due to the binary interaction between zeolite and nano-potassium. There was a substantial influence of the triple interaction between the study components on the chlorophyll content of the leaves, with the greatest averages being found in the BZ1K2 and BZ0K0 treatments, with values of 1.752 and 1.681 mg gm^{-1}, respectively.1.567 mg gm^{-1}.

Table 4. Effect of adding zeolite and spraying with nano-potassium and their interactions on the content of chlorophyll in leaves (mg gm^{-1} fresh weight) of two types of radish.

Radish type (T)	Zeolite (Z) t ha^{-1}	Nano-Potassium (K) gm L^{-1}			Interaction	T Mean
		0 (K0)	1(K1)	2 (K2)	Z × T	
Black (B)	0 (Z0)	1.654	1.654	1.658	1.655	1.682
	10 (Z1)	1.681	1.674	1.752	1.702	
	20 (Z2)	1.660	1.658	1.657	1.658	
Red (R)	0 (Z0)	1.597	1.665	1.646	1.626	1.647
	10 (Z1)	1.650	1.645	1.669	1.654	
	20 (Z2)	1.651	1.628	1.672	1.650	
K Mean		1.643	1.654	1.675	L.S.D 0.05	L.S.D 0.05
L.S.D 0.05T × Z × K			0.089		Z × T 0.051	T 0.029
Interaction	Black	1.665	1.662	1.689	L.S.D 0.05T × K 0.051	

Radish type (T)	Zeolite (Z) t ha^{-1}	Nano-Potassium (K) gm L^{-1}			Interaction Z × T	T Mean
		0 (K0)	1(K1)	2 (K2)		
T × K	Red	1.632	1.646	1.662	Z mean	
Interaction Z × K	0 (Z0)	1.625	1.660	1.652	1.645	
	10 (Z1)	1.665	1.660	1.710	1.688	
	20 (Z2)	1.655	1.643	1.664	1.654	
		L.S.D 0.05				
	Z × K		K		Z	
	0.063		0.036		0.036	

3.5. Leaves Content of Total Carbohydrates %

The addition of zeolite and the spraying of potassium nanoparticles did not have a statistically significant effect on the carbohydrate content of the leaves (Table 5), but there were statistically significant differences between the two types of radish (Table 6). The black radish averaged 11.53% more total carbohydrates than the red radishes did (9.75%). Treatment BZ2, which produced an increase in carbohydrate content of 11.57%, was found to be significantly superior to treatment RZ0, which produced an increase of only 9.23%, according to the results of a two-way interaction between zeolite treatment and radish type. Bilateral interaction between nano-potassium treatments and radish type is also displayed in the table; for example, treatment BK2 performed best and produced the highest percentage of carbs (11.57%), while treatment RK0 produced the lowest percentage of carbohydrates (9.34%). Treatment Z1K1 recorded the highest percentage of carbohydrates, totaling 10.97%, which was equal to treatment Z2K2, so it gave the same percentage compared to treatment Z0K1, which recorded the lowest percentage of carbohydrates, totaling 10.29%, demonstrating the superiority of the interaction of zeolite with nano-potassium. Results from the study's triple interaction between components indicated that treatment BZ1K1 produced the highest percentage of carbs (11.61%) compared to treatment RZ0K1, which produced the lowest percentage of carbohydrates (9.12%).

Table 5. Effect of adding zeolite and spraying with nano-potassium and their interactions on the leaves content of Carbohydrates % of two types of radish.

Radish type (T)	Zeolite (Z) t ha^{-1}	Nano-Potassium (K) gm L^{-1}			Interaction Z × T	T Mean
		0 (K0)	1(K1)	2 (K2)		
Black (B)	0 (Z0)	11.33	11.47	11.54	11.45	11.53
	10 (Z1)	11.54	11.61	11.54	11.56	
	20 (Z2)	11.48	11.56	11.58	11.57	
Red (R)	0 (Z0)	9.37	9.12	9.21	9.23	9.75
	10 (Z1)	9.31	10.33	10.35	9.99	
	20 (Z2)	9.35	10.35	10.36	10.02	
K Mean		10.41	10.74	10.76	L.S.D 0.05	L.S.D 0.05
L.S.D 0.05T × Z × K			0.952		Z × T 0.11	T 0.06
Interaction T × K	Black	11.48	11.55	11.57	L.S.D 0.05T × K 0.11	
	Red	9.34	9.93	9.97	Z mean	
Interaction Z × K	0 (Z0)	10.35	10.29	10.37	10.34	
	10 (Z1)	10.42	10.97	10.94	10.78	
	20 (Z2)	10.46	10.95	10.97	10.80	
		L.S.D 0.05				
	Z × K		K		Z	
	0.13		0.07		0.07	

Tables (1-5) indicate a clear effect of all treatments that contain zeolite on all the studied characteristics of plant height, number of leaves, leaf area, leaf chlorophyll content and percentage of carbohydrates in leaves and that may be attributed to the fact that the mineral zeolite has a positive effect on the physical, chemical and biological properties of the soil, directly or indirectly, which leads to an increase in the ability of the soil to retain nutrients and thus improves plant growth, and this was

confirmed by[21]. The plant that grows in soil to which zeolite is added produces a strong root system deep in the soil, which causes an increase in the efficiency of water absorption and nutrients during the plant growth period, and thus increases their vital metabolism within the plant, encouraging plant growth and that the mineral works to provide elements adsorbed on the surface of the mineral and its internal structure, which will affect the readiness of nutrients in the soil such as nitrogen, phosphorus, potassium, iron, magnesium and give them to the plant gradually when it needs them, and thus the cells multiply by increasing their division and elongation [22]. Zeolite mineral protects the conversion of nitrogen from NH_4^+ to NO_3^+ by the nitrification process, thus preserving the ion from leaching through the soil that leads to groundwater pollution, as well as its permanent and gradual availability [23]. The results of Al-Ibraheemi [24] when using zeolite for lettuce plants confirm what was reached in this study, where treatments containing zeolite caused a significant increase in all vegetative traits of the plant. Spraying with nano-potassium alone did not have a significant effect except on the characteristic of plant height at a concentration of 2 gm L^{-1}, but it had a significant effect at the double and triple interference an increase in most of the studied traits (Tables 1-5), which can be attributed to the positive relationship between the mineral and potassium, as potassium has an important role in transporting the products of the photosynthesis process, which affects the vital processes by activating the enzymes that are related to the energy transfer processes and thus causes an increase in photosynthesis [25].The presence of potassium in a balanced manner with other nutrients such as nitrogen, phosphorus, magnesium, calcium helps to form a large and strong root system, which increases the process of absorbing nutrients, stimulates cell division and increases the process of photosynthesis, which is reflected in an increase in plant growth and improving the quality and quantity of the yield [26,27]. The results showed the superiority of black radish over red radish in all studied traits, whether singly or in combination with the rest of the study factors (Tables 1-5). This may be due to the genetic factors that control the absorption and accumulation of nutrients within the plant, and thus the plants' response to the fertilization programs that are applied to improve the quantitative and qualitative production varies [21].

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Impact of Benzyl Adenine (BA) on the Propagation of *Dianthus Chinesis in Vitro*

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Abstract. The experiment was carried out in plant tissue culture laboratory of Department of Horticulture and Landscape Engineering - College of Agriculture - Tikrit University during the academic year (2022-2023) on *Dianthus chinesis*. Results were analyzed according to the order of the complete random design R.C.D and with 10 replicates and under a probability level of 0.05, where the results of the statistical analysis to study Impact of different concentrations of Benzyl Adenine (BA) on Doubling and doubling of tips of branches and single nodes of Chinese carnation plant, it was found that the concentration is 1.0 mg It was superior to the rest of the concentrations in the stages of development and Doubling, as it gave highest number of leaves and highest number of branches, while in the length of branches there was no significant difference when treated with concentration 1.0 mgL^{-1} and treated with concentration 0.5 mgL^{-1}. The impact of different concentrations of IBA and the concentration of medium salts on the rooting of branches resulting from Doubling was also studied. It was shown that the highest 60% root rate was obtained at the 2.0 concentration of IBA and the use of the middle of MS with half the concentration of salts compared to the same focus of IBA on the entire concentration. The highest rooting percentage was 50%.

Keywords. Dianthus Chinesis, Tissue Culture, BA.

1. Introduction

The Chinese Carnation is one of most widely known Ornamental plants [1]. Belongs to the caryophyllaceae family. Its stems are straight and its leaves are ribbon-shaped, and its flowers differ in their colors and shapes Its height is 50 cm or a little more [2]. The original home of this plant is Maluku Islands in Indonesia and southern Philippines, but today its cultivation is widely spread in several countries, including Brazil, Madagascar and the Andes Mountains [3].

Because different types of Carnations contain many active substances, especially flavonoids, which makes them have many medical benefits, they are known for their activity as antioxidants, anti-inflammatory, liver protection and anti-many disease, especially age-related [4,5].

Plant tissue culture technology is one of the biotechnologies used to stimulate genetic variation to improve plant qualities [6]. This technology is defined as the cultivation of a cell or plant organ in a sterile nutrient medium under controlled laboratory conditions [7].

Plant growth regulators are organic compounds, but not nutrient they may be natural or artificial, they change the development and growth of plants and they may stimulate or inhibit plant growth [8].

Growth regulators play a major role in determining the goal to be reached from tissue culture, especially the formation processes that occur outside the living body [9].

The emergence stage is one of the important stages of propagation, which has significant Impacts on the success or failure of the Doubling process , the quality and number of the resulting plants depend on it, which indicates the possibility of choosing the appropriate medium that achieves the best speed of growth and multiplication and it has multiple directions, including doubling the number of plants through breaking the apical dominance of the tips of the branches or through growth of the adventitious branches or through the differentiation of the callus branches [10-12]. The Doubling phase is one of the important stages where at this stage the success or failure of the prpagation process is determined, as at this stage the vegetable part that was cultivated in the Doubling phase is abundant [13]. Overlap between the vegetable Part and the type of cytokinins used plays an important role in the extent of response to the Doubling. Many researchers have shown the large role of developing peaks in multiplying, when using either cytokinins alone or overlapping with auxins. The activation of cytokinins used to double plant parts is BA benzyl adenin [14].

Possibility of multiplying two varieties of *Dianthus caryophyllus* has been studied, The first was Marie Chaboud Jaune and the second was Jeanne Doinis Blano, where its response to tissue culture was 100%, The best branch Doubling was obtained when growing the developing peaks of the white floral class(Jeanne Doinis Blano) on the MS, which was equipped with 8 mgL^{-1} of Kin, with 16.90 branches per vegetable part, while 14.40 branches per vegetable segment were used when using the concentration of 6 mgL^{-1} of Marie Chaboud Jaune after eight weeks[15]. Developing peaks of *Dianthus caryophyllus* of white and yellow floral varieties, whose response to tissue culture was 100%, multiply with 23.10 branches of yellow varieties per vegetable portion at the concentration of 3 mgL^{-1} of benzyl adenin [16].

Rooting stage is an important stage of micropropagation in the plant outside the living body, as it stimulates the branches resulting from the Doubling stage to produce roots during this stage, or the next stage (In Vivo) and when the branches were transferred to the rooting medium, good results were obtained, as it took the plants (2-4) weeks to root [17]. The nutrient medium was equipped with auxins due to their importance in the emergrnce and development of adventitious roots [18].

Rooting percentage of vegetative branches that were produced from the carnation leaf callus *Dianthus caryophyllus* L. on MS medium with a concentration of 1.0 mgL^{-1} of IBA reached 100%, where the increase in the number of roots was an average of 6.6 roots and root length. An average of 4.8 cm after 9 days of planting the vegetative branches [19]. Highest rooting percentage was obtained by 98.19% when growing the tips of the *Dianthus caryophyllus* L. which was produced from tissue culture on MS medium with half salt concentration provided with a concentration of 1.5 mgL^{-1} of IBA and 0.02% of activated charcoal. Roots at a rate of 9.60 roots per vegetable Part and root length at an average of 4.24 cm [20].

2. Materials and Methods

2.1. Nutritional Medium and its Preparation

The MS medium produced from the Indian company (HIMEDIA) recommended by [21]. Was used with the addition of some growth regulators and according to the objective of the study. Stock solutions were prepared from the used plant growth regulators and kept in the refrigerator at a temperature of 4°C in glass beakers and to prepare one liter of the medium, distilled water was placed in a glass beaker on a hot rotating hot plate magnetic stirrer and 10 g of agar-agar was added to it until boiling and after it had completely dissolved, 4.43 gm was added to it. From the MS medium and finally 30 g of sucrose was added, then the volume was completed to 1000 ml using a cylinder Cylinder to add the plant growth regulators according to the objective of the study and the PH was adjusted PH 5.8 ± 0.1 either by adding drops of 1 N NaOH or drops of hydrochloric acid 1 N HCl and measured using a pH meter. Then the nutritional medium was poured into glass bottles of 100 ml capacity of 20 ml for each bottle, and the nozzles of these bottles were covered with heat-resistant aluminum foil.

2.2. Sterilization of Food Medium

After pouring the nutritional medium into 100 ml glass bottles, they were sterilized using an autoclave at a temperature of 121°C and a pressure of 1.04 $kgcm^{-2}$ for 20 minutes. After the sterilization period ended, the bottles were taken out and left in the development room to cool until the medium solidified. With this, it is ready for cultivation.

2.3. Development Conditions for Plants

Cultures of the propagation experiments were incubated in the Incubator at a temperature of 25±2°C, illumination intensity 3000 lux, 16 hours light succession followed by 8 hours of darkness daily. They were prepared from white fluorescent tubes.

2.4. The Source of the Plant Parts

The plant parts were obtained from tissue mother cultures resulting from planting the seeds of *Dianthus chinesis* class Godetia , where the flowers of this class are distinguished by their pink color (obtained from the local markets, the producing company IKLIM BAHCE LTD STI) on nutrient medium free of plant growth regulators and after 6-7 weeks the plant parts were taken from them:

- Shoot tips: They were used for purpose of emergence and multiplication.
- Nodes: They were used for purpose of emergence and multiplication.
- The resulting branches of multiplication: They were used for the purpose of rooting.

2.5. Surface Sterallization of Seeds

The seeds of *Dianthus chinesis* were washed with running water for an hour to remove the remnants of dust and dirt, to be ready for sterilization and then sterilized with 70% ethyl alcohol for 30 second, then washed with distilled water and then transferred to the planting table (cabinet) to be sterilized with a solution. Sodium hypochlorite NaOCl 15% for 10 minutes (this solution containing 6% of sodium hypochlorite) by placing the seeds in a glass container of 250ml, adding the sterilization solution , covering the glass container and doing continuous stirring to the sterilization period ends, after which the sterilization solution is poured, the seeds are washed with sterilized distilled water 3 times for 5 minutes each time to remove the harmful effect of the sterilized material. Thus, the seeds become ready for cultivation, as they were grown on MS medium free of growth regulators and then transferred to the incubator.

2.6. Stages of Micropropagation

2.6.1. Stage of Emergence and Doubling

The stage of Emergence the different plant parts, whether the growing tops or the nodes are cultivated on MS medium equipped with different concentrations of growth regulators, where it is provided with concentrations(0, 0.5 , 1, 1.5 , 2 mgL^{-1} of BA) As for the Doubling stage, these parts (growing tops and nodes) were planted on the same media that were planted on them in the budding stage and for another four weeks, after which data on the growth and development of the plant parts were recorded at the end of each stage, when planting the parts The plants were incubated for 4 weeks in each stage, with 10 replicates for each concentration.

The Characteristics that have been studied in Emergence Stage :

- Average number of branches (branch / plant part).
- average length of longest branch (cm).
- Average number of leaves (leaf / plant part).

The studied Characteristics of the Doubling Stage as an average :

- Number of branches (branch / vegetable part).
- length of the longest branch (cm).
- Number of leaves (leaf for / plant part).

2.6.2. Rooting Stage

- Rooting on MS medium (half salt concentration): Branches that resulted from the Doubling were grown on MS medium (half salt concentration) equipped with different concentrations of IBA growth regulator (0, 0.5, 1, 1.5, 2 mgL^{-1}) for rooting and Data were taken 4 weeks after transplantation.
- Rooting on MS medium (half salt concentration): Branches that resulted from the Doubling were grown on MS medium (full salt concentration) equipped with different concentrations of IBA growth regulator (0, 0.5, 1, 1.5, 2 mgL^{-1}) for rooting and Data were taken 4 weeks after transplantation.

2.6.3. The Characteristics that were Studied in the Rooting Stage

- Percentage of Rooting (%) .
- Average number of roots (root / plant part).
- Average length of the longest root (cm).
- Average number of leaves (leaf / plant part).

2.7. Design and Analysis of Experiments

The results were analyzed in a C.R.D. 10 tubes (Repeats) were taken randomly, where each replicate contained one plant part, and the rates were compared on Duncan's polynomial test under a probability level of 5%, and the ready-made program SAS (2001) was used to analyze the data [22].

3. Result and Discussion

3.1. Emergence Stage

3.1.1. Tips of Branches

Results of Table (1) Show that there is a significant Impact when adding different concentrations of Benzyl Adenine on the studied Characteristics after 4 weeks of cultivation, where the treatment with concentration 1.0 mgL^{-1} of Benzyl adenine gave the highest average number of leaves of 24.7 leaves/vegetable part compared to the lowest value Which reached 6.5 leaves/vegetable fraction when 2 mgL^{-1} of BA was added to the medium.

As for the Number of Branches, the treatment with a concentration of 1.0 mg / liter gave the highest average number of branches, which amounted to 3.7 branches / plant part, compared to the lowest value, which amounted to 1.0 branch / plant part, in which all other concentrations (2.0, 1.5, 0.5, 0.0) participated. mgL^{-1} , as there was no significant difference between them.

It also had the longest Branch length trait, highest mean of the longest branch length was 3.6 cm in relation to the concentration 1.0 mgL^{-1} , which did not differ significantly when treated with the concentration 0.5 mgL^{-1} , which was 3.5 cm, compared to the concentration 2.0 mgL^{-1} , which gave the lowest value an average of 1.4 cm. Figure (1) Shows the Impact of treatment with a concentration of 1.0 mgL^{-1} and a concentration of 2.0 mgL^{-1} of BA on the Doubling of branch tips.

Table 1. Impact of Different Concentrations of Benzyl Adenine (BA) on the Growth of Tips of the Branches of Chinese Carnation.

BA Concentrations (mgL^{-1})	leaves Number (leaf/ Vegetable Part)	Branches Number Branch/ Vegetable) Part)	Branch Longest length (cm)
0.0	9.5 b	1.0 b	2.5 b
0.5	9.4 b	1.0 b	3.5 a
1.0	24.7 a	3.7 a	3.6 a
1.5	8.0 b	1.0 b	1.7 bc
2.0	6.5 b	1.0 b	1.4 c

*Means with similar letters have no significant difference between them according to Duncan's polynomial test at 5% probability level.

Concentration 1.0 **Concentration 2.0**

Figure 1. Impact of Different Concentrations of BA on the growth of the tips of the branches of Chinese carnation plant for the highest value and the lowest value.

3.1.2. Single Nodes

The results of Table (2) show that there is a significant Impact when adding different concentrations of benzyl adenine on the studied Characteristics, where the treatment with the concentration of 1.0 mgL^{-1} of benzyl adenine gave the highest average number of leaves of 17.9 leaves/vegetable part compared to the lowest value, which amounted to 10.4 leaves/ Vegetarian fraction when 2 mgL^{-1} of BA is added to the medium.

As well as for the characteristic of the number of branches, the treatment with a concentration of 1.0 mgL^{-1} gave the highest rate of the number of branches, which reached 3.1 branches / plant part, compared to the lowest value, which amounted to 1.0 plant branches/part, in which the concentrations (2.0, 1.5, 0.0) mgL^{-1} participated. There was no significant difference between them.

As for the longest branch length trait, the highest mean of the longest branch length was 2.9 cm in relation to the concentration 0.5 mgL^{-1} , which did not differ significantly when treated with the concentration 1.0 mgL^{-1} , which was 2.6 cm, compared to the lowest value when treated with the concentration 0.0 mgL^{-1} Which is 1.5 cm.

Table 2. Impact of Different Concentrations of Benzyl Adenine (BA) on the Doubling of 2-Single Nodes of Chinese carnation.

BA Concentrations (mgL^{-1})	leaves Number (leaf/ Vegetable Part)	Branches Number Branch/ Vegetable Part)	Branch Longest length (cm)
0.0	11.9 ab	1.0 b	1.5 b
0.5	11.9 ab	1.4 b	2.9 a
1.0	17.9 a	3.1 a	2.6 a
1.5	14.7 ab	1.0 b	1.6 b
2.0	10.4 b	1.0 b	2.0 b

3.2. Doubling Stage

3.2.1. Tips of the Branches

The results of Table (3) Showed a significant Impact when adding different concentrations of benzyl adenine on the doubling of the tips of the branches after 8 weeks of planting, where the treatment with the concentration of 1.0 mg / L of benzyl adenine gave the highest average number of leaves of 72.0 leaves / vegetable part compared to the lowest A value of 12.7 leaves/vegetable portion when 2 mgL^{-1} of BA was added to the medium.

As for the characteristic of the number of branches, the treatment with the concentration of 1.0 mg / liter gave the highest rate of the number of branches, which reached 8.7 branches / plant part, compared to the lowest value, which amounted to 1.0 plant branch / part when treated with the two concentrations (2.0,0.0) mg / liter where there was no Significant difference between them.
Also for the longest branch length trait, the highest mean of the longest branch length was 4.4 cm in relation to the concentration of 0.5 mgL^{-1} , which did not differ significantly when treated with the concentration of 1.0 mgL^{-1} , which amounted to 4.0 cm, and the concentration of 1.5 mgL^{-1} , which amounted to 3.6 cm. Compared to the lowest value when treated with a concentration of 2.0 mgL^{-1} , which was 2.2 cm. Figure (2) shows the Impact of treatment with a concentration of 1.0 mgL-1 and a concentration of 2.0 mgL-1 of BA on the doubling of the tips of the branches.

Table 3. Impact of Different Concentrations of Benzyl Adenine (BA) on Doubling of the Tips of Branches Clove plant.

BA Concentrations (mgL^{-1})	leaves Number (leaf/ Vegetable Part)	Branches Number Branch/ Vegetable Part)	Branch Longest length (cm)
0.0	13.1 c	1.0 b	3.5 ab
0.5	20.0 bc	1.9 b	4.4 a
1.0	72.0 a	8.7 a	4.0 a
1.5	32.1 b	2.1 b	3.6 a
2.0	12.7 c	1.0 b	2.2b

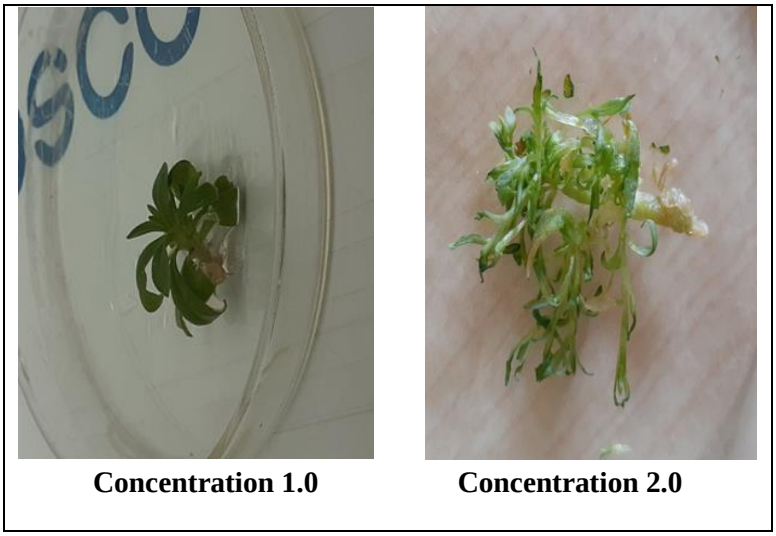


Figure 2. Impact of Different Concentrations of (BA) on the Doubling of the Tips of Branches of Chinese Carnation for the Highest Value and Lowest Value .

3.2.2. Single Nodes

Results of Table (4) show a significant Impact when adding different concentrations of benzyl adenine on the Doubling of single nodes. The treatment was characterized by the concentration of 1.0 mgL^{-1} of benzyl adenine with the highest average number of leaves of 50.6 leaves/vegetable part compared to the lowest value of 9.9 leaves. A plant fraction when treated with a concentration of 2 mgL^{-1} of BA.

As for the number of branches, the treatment with the concentration of 1.0 mgL^{-1} gave the highest average of the number of branches, which was 5.9 branches/plant part, compared to the lowest value, which amounted to 1.0 branch/plant part, which was obtained from the treatment with the concentration of 0.0 mgL^{-1} .

While the characteristic of the length of the longest branches, the highest value for the length of the longest branch reached 4.4 cm in relation to the 0.0 mgL^{-1} concentration, compared to the lowest value when treated with the 1.5 mgL^{-1} concentration, which was 2.4 cm.

Table 4. Impact of Different Concentrations of Benzyl Adenine (BA) on the Doubling of Single Nodes of *Dianthus chinesis*.

BA Concentrations (mgL^{-1})	leaves Number (leaf/ Vegetable Part)	Branches Number Branch/ Vegetable Part)	Branch Longest length (cm)
0.0	20.9 bc	1.0 b	4.4 a
0.5	28.0 b	1.8 b	3.4 ab
1.0	50.6 a	5.9 a	3.8 ab
1.5	21.4 bc	2.1 b	2.4 b
2.0	9.9 c	1.4 b	3.3 ab

3.3. Rooting Stage

3.3.1. Rooting on MS Medium with Half Salt Concentration

The results of Table (5) show a significant Impact when using different concentrations of indole butyric acid IBA in addition to using a medium with half the salt concentration on the rooting of the branches resulting from the doubling. The highest rooting percentage reached 60% when treated with concentration 2.0 mgL^{-1} compared to the lowest percentage and Which amounted to 10% when treated with a concentration of 0.0 mgL^{-1} on MS medium with half the salt concentration.

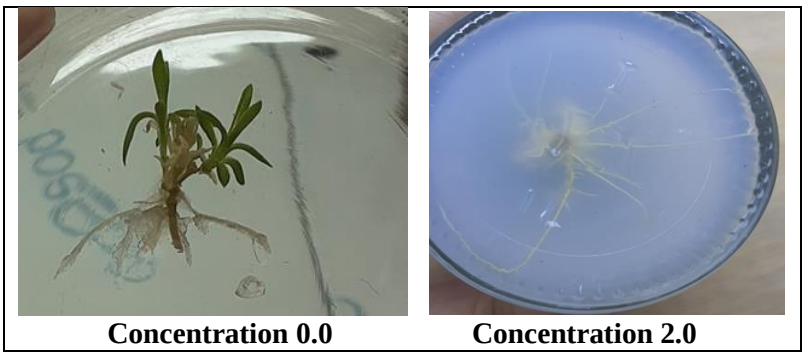
Also, treatment with a concentration of 2.0 mgL^{-1} of IBA was characterized by the highest rate of root number with a value of 4.6 root/vegetable part compared to the lowest value which was 0.3 root/ vegetable part when treated with a concentration of 0.0 mgL^{-1} on MS medium with half salt concentration.

The Average length of the longest root, the treatment with concentration of 2.0 mgL^{-1} also gave the highest rate, which was 1.1 root/vegetable part compared to the lowest value which was 0.1 root/ vegetable part when treated with concentration of 0.0 mgL^{-1} .

As for the number of leaves, the highest rate of the number of leaves was 25.0 leaves / vegetable part for the concentration of 2.0 mgL^{-1} compared to the lowest rate of the number of leaves when treated with the concentration of 0.5 mgL^{-1}, 7.3 leaves / vegetable part were obtained, Figure (3) shows Impact of treatment with concentration of 2.0 mgL^{-1} and concentration of 0.0 mgL^{-1} of IBA and concentration of medium salts on rooting of branches.

Table 5. Impact of Different Concentrations of Indole Butyric Acid (IBA) in Rooting Branches of *Dianthus chinesis* on MS medium with Half Salt Concentration.

IBA Concentrations (mgL^{-1})	Rooting Percentage %	Number of roots (Root vegetable part)	Longest Root length (cm)	Number of Leaves (leaf/vegetable part)
0.0	10 b	0.3 b	0.1 b	10.6 c
0.5	20 ab	1.7 ab	0.2 b	7.3 c
1.0	30 ab	2.1 ab	0.4 ab	11.4 c
1.5	30 ab	2.4 ab	0.5 ab	15.8 b
2.0	60 a	4.6 a	1.1 a	25.0 a



Concentration 0.0 **Concentration 2.0**

Figure 3. Shows the Impact of Different Concentrations of IBA on Rooting of Branches Resulting from Doubling on MS Medium with Half the Salt Concentration.

3.3.2. Rooting on MS Medium with Full Salt Concentration

Table (6) indicated that there is a significant Impact when adding different concentrations of indole butyric acid IBA to MS medium with full salt concentration on rooting of branches resulting from doubling. The highest rooting percentage reached 50% when treated with concentration 2.0 mgL^{-1} compared to the lowest percentage which It reached 0% when treated with a concentration of 0.0 mgL^{-1} on MS medium with full salt concentration.

Also, treatment with a concentration of 2.0 mgL^{-1} of IBA was characterized by the highest rate of root number by 13.7 roots/ vegetable part compared to the lowest value which was 0.0 root/ vegetable part when treated with a concentration of 0.0 mgL^{-1} on MS medium with full salt concentration. Average length of the longest root, treatment with concentration of 2.0 mgL^{-1} also gave the highest rate, which was 1.6 root/plant part, compared to the lowest value, which was 0.0 root/vegetable Partwhen treated with concentration of 0.0 mgL^{-1} .

As for the number of leaves, the highest average number of leaves was 20.0 leaves/vegetable Partfor the concentration 2.0 mgL^{-1} compared to the lowest rate for the number of leaves when treated with the concentration 0.0 mgL^{-1}, 7.1 leaves/vegetable Partwere obtained, Figure (4) shows Impact of treatment with concentration of 2.0 mgL^{-1} and concentration of 0.0 mgL^{-1} of IBA and concentration of medium salts on rooting of branches.

Table 6. Impact of Different Concentrations of Indole Butyric acid (IBA) in Rooting Branches of *Dianthus chinesis* on MS medium with Full Salt Concentration.

IBA Concentrations (mgL^{-1})	Rooting Percentage %	Number of roots (Root vegetable part)	Longest Root length (cm)	Number of Leaves (leaf/vegetable part)
0.0	0 b	0.0 b	0.0 b	7.1 d
0.5	10 b	0.8 b	0.2 b	11.8 c
1.0	10 b	1.5 ab	0.3 ab	14.7 bc
1.5	30 ab	9.7 ab	0.9 ab	18.1 ab
2.0	50 a	13.7 a	1.6 a	20.0 a

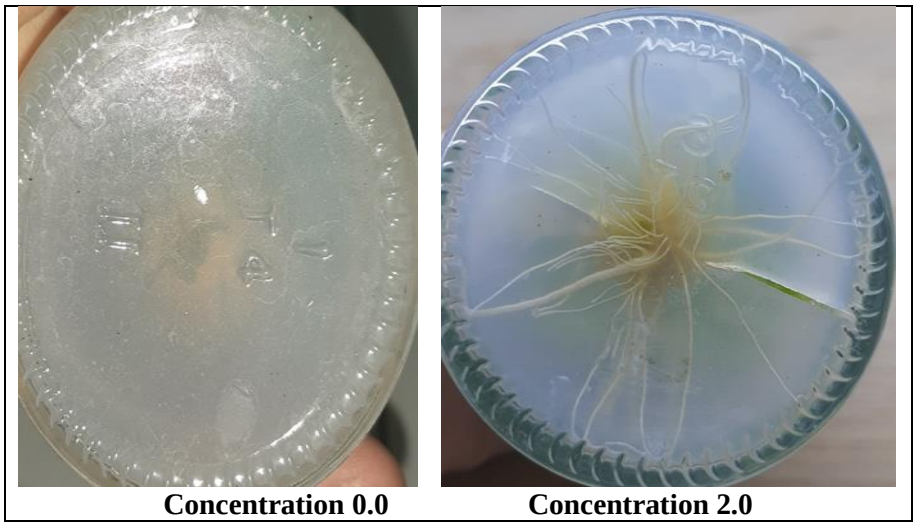


Figure 4. Shows the Impact of Different Concentrations of (IBA) on Rooting of Branches Resulting from Doubling on MS medium with Full Salt Concentration.

Conclusion

With the same emphasis on IBA on the full concentration, the maximum 60% root rate was obtained at the 2.0 concentration of IBA and the use of the middle of MS with half the concentration of salts. There was a maximum of 50% rooting success.

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Isolation and Identification of a Novel Strain of Levansucrase Producer Bacteria named *Bacillus lichniformans* MJ8 and Synthesis Levan

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Abstract. The ability to produce levansucrase was tested in fifty isolated, purified Bacillus spp. from various sources. It was found that the eleven isolates were the higher producers of this enzyme through a primary screening determined by the amount of sticky mucous membrane formed around the colonies, demonstrating their capacity to produce levan, a polysaccharide formed by levansucrase. All producer isolates were subjected to further cultural and morphological analysis to confirm that they belong to Bacillus sp. The secondary screening was performed on these isolates by estimation levansucrase assay, and it was found that the isolate Bacillus sp. S-8 was the best producer, as its activity reached 6.05 U/ml. The Vitek2 compact system was used to analyze the biochemical tests to identify this isolate. This was followed by PCR and nitrogen-base sequencing to identify the 16S ribosomal RNA gene. The results showed that this isolate is Bacillus licheniformis, designated in this study as MJ8. This bacterium was registered in GeneBank under the Accession Number OM672244.1. The levan synthesis by these bacteria was identified and characterized utilizing the techniques of HPLC, FTIR, SEM, and AFM analysis.

Keywords. 16S rRNA, Bacillus, GeneBank, Vitek2, PCR, HPLC.

1. Introduction

Levansucrase (E.C. 2.4.1.10) builds levan, a glycoside hydrolase belonging to family 68, from fructose residues. Levansucrase hydrolyzes sucrose, releases glucose, and adds fructose molecules to an expanding levan fructooligosaccharide (L-FOS) chain [1]. The fructooligosaccharide, also known as levan, is a common homopolysaccharide made primarily of fructose units by β-(2→6) glycosidic linkage in the main chain and β-(2→1) linkage at branch points. Plants and microorganisms produce levan, such as numerous algal cells, yeasts, fungi, and bacteria [2]. Levan may help bacteria and plants resist biological stresses by regulating cell osmotic pressure and enhancing drought resistance, salinity resistance, and low-temperature protection, according to earlier studies [3,4]. Levan's practical and essential qualities also make it applicable in various industries. Jakob et al. [5] discovered that levan creates a hydro-colloid microgel that can be added to wheat bread to increase product shelf life. Ragab et al. [6] said levan could be important for peptic ulcer problems. Levan's prebiotic properties have been confirmed by several additional studies [7,6], as well as it is antioxidant [8], anti-obesity [9], anti-fungal [10], anti-diabetic and anti-tumor [8] properties. Researchers keep enhancing the yield and

quality of levan because of its exceptional properties and wide range of potential applications. This study aimed to isolate a *Bacillus* species that can produce high levansucrase activity from various sources in Baghdad, Iraq. Also included in the study is an enzymatic synthesis of levan, separation, purification, and identification using various techniques. This was done because there have been few studies on the production of this polymer in Iraq.

2. Materials and Methods

2.1. The Isolation Sources

Rancid jam, remnants of natural juice, sweets on vendor carts that were ready for consumption, samples of rotten fruit, and soils were some sources of isolation. These samples were collected from various locations throughout Baghdad. Ten grams of each sample were suspended in distilled water with a pH of 7.0, then heated for 10 minutes at 80 °C to destroy the vegetative cells of the bacteria. 20 ml of nutrient agar with 1 ml of suspension was then kept overnight at 37 °C. The isolated colonies that exhibited the morphological characteristics of a *Bacillus* species were chosen, screened, and identified.

2.2. Morphological Characterization of Isolation

Bergey's Manual of Systematic Bacteriology Is a taxonomic key used to identify isolates based on their cultural and morphological traits [11].

2.3. Primary Screening

The purity isolates were subjected to primary screening using the streaking method on the mineral salt agar- with 20% sucrose described by Radhi and Hasan [12] and incubated for 48 hours at 37°C. The extraordinarily sticky mucous membrane produced around bacterial colonies demonstrated the ability for levansucrase production.

2.4. Secondary Screening (Levansucrase Production)

According to Shih et al. [13], the isolates of *Bacillus* spp. that passed the primary screening were activated; the 2 ml of bacterial inoculum containing 1.5 X 10^8 cells/ml (McFarland stander curve O.D. 600) and were subjected to produce levansucrase using submerged culture fermentation by mineral salt broth with 20% sucrose. The flask fermentation was incubated in a shaker at 150 rpm for 48 hours at 37 °C. After that, the cultures were filtered by centrifuging them at a speed of 10,000 x g for 20 min at 4 °C, and the clear supernatant was used to determine the enzyme activity (U/ml) for all isolates.

2.5. Levansucrase Activity Assay

The enzyme activity was determined according to Pongsakorn et al. [14] with some modifications. 0.5 ml of the substrate (5% sucrose (W/V) sodium phosphate buffer 0.05M, pH 7.0) and 0.5 ml of crude enzyme and incubated at 37 °C for 10 minutes. Then 1 ml of (DNS) was added to stop the reaction and heat for 5 min. The absorbance was measured at 540 nm. Standard glucose was achieved as a calibration curve, according to [15]. One unit of the enzyme was defined that produced 1 µml of glucose/min under stander conditions.

2.6. Identification of Selected Isolate (S-8)

2.6.1. Vitek2 Compact System Identification

The specialized diagnostic kit for the Bacillaceae family (BCL), which contains 46 biochemical tests, was used to identify the isolate (S-8). The procedure was followed according to the manufacturer's instructions, BioMerieux [16].

2.6.2. Identification of the 16S Ribosomal RNA Gene

The DNA of *Bacillus* Sp. Isolate (S-8) was extracted using a DNA extraction kit (Geneaid Co., Taiwan) and amplified using primers according to Bassem et al. [17] (5'AGAGTTTGATCCTGGCTCAG3') and reverse (5'TACGGYTACCTTGTTACGACTT3') by PCR. Amplification by PCR with a first denaturation step of 95 °C for 5 min, then 35 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min, and finally a final extension step of 72 °C for 7 min [18]. The result of PCR was relocated in 1% Agarose gel for 45 minutes at 90V while loaded with a 2 µl DNA ladder with a 1500 bp. The DNA sequences were determined by (Macrogen Co., Korea) and analyzed by BLAST from the (NCBI), with MEGA11 software 11.0.1.

2.7. The Polymers Levan Biosynthesis, Separation, and Purification

The biopolymer was produced from the local isolation using the submerged culture method described by Shih et al. [13] and incubated in a shaker incubator at 150 rpm, 37°C for 48 hours. After incubation, the levan was extracted using the Srikanth et al. [19] method. The cells were heated for 30 minutes at 100°C and then allowed to cool; for 30 minutes, centrifugation at 10,000 x g produced two layers of supernatant and sediment. For five minutes, the supernatant was placed back in the water bath. And then allowed cooling at 25°C to remove the extracellular enzymes' remaining efficacy. Using potassium hydroxide 1M KOH, the pH of the supernatant was then adjusted to be between 9.0 and 10.0. Following that, -20 °C, 80% concentration, and 1:2 (volume of solution to the volume of extraction solvent) chilled ethanol were added. It was then given 1 ml of 1% $CaCl_2$ with continuous stirring for 20 minutes to speed up the levan precipitation process, and it was kept the following day at 4 °C to give it enough time to precipitate. The precipitated granules were collected by centrifugation at 10,000 x g for 15 min, washed once more with chilled ethanol at a concentration of 80% at a rate of 1:4 (weight: volume of extraction solvent), and then put in a pre-prepared sterile glass petri dish and allowed to dry at 45 °C. Following that, the purification stage was carried out in accordance with the procedure outlined by Bajpai et al. [20]. After drying with de-ionized water (DIW), the dried material was redissolved to produce the levan. Then, to thoroughly remove any detectable low molecular weight proteins, nuclear acids, or other organic compounds, a dialysis process was carried out using bags with a pore diameter ranging from 12 to 14 kDa against deionized water and left at 4 °C for 72 hours [21]. Subsequently, a lyophilization operation at -55°C was performed using (Alpha 1-2 LD lyophilization, Christ Co., GERMANY) to obtain a dry powder, which was stored at 4 °C until the required examinations were carried out.

2.8. The Polymers Identification Structure

2.8.1. Identification of Polymer by HPLC

The USA HPLC system FLC (Fast Liquid Chromatographic) NH2 column (50×4.6 mm) 3 µm was used to conduct this analysis. A reflective index detector separated the aqueous extract for I.D. detection (Shimadzu RID-10A). The rate flow for the mobile phase is 0.1 mL/min containing deionized water in 50 volumes with 50 volumes of acetonitrile at 30 °C. The injection sample 20µl included, in addition to the levan produced in this study, two standard levan, one produced from the bacteria *Erwinia herbicola* (SKU number L8647-1G) and another from Chicory (SKU number F8052-50G), both supplied by (Sigma-Aldrich Co., Germany) and also included in the standard (fructose, glucose, and sucrose) from (HiMEDIA Co., INDIA) The standards were prepared by dissolving a sufficient amount of the sugars in (10 ml of the mobile phase 100 µg/ml and then diluted to 25 µg/ml) and filtered through Millipore with a 0.45-µm pore size membrane; The separation process was carried out sequentially under the optimal conditions of the experiment [22].

2.8.2. Identification of Polymer by FTIR

The Bruker–Tensor 27 with an ATR unit was used to conduct this analysis. The levan was compared to the levan from *Erwinia herbicola* (Sigma-Aldrich Co., GERMANY) as a standard. The device calculates the amount of infrared energy that passes through a sample in the 600–4000 cm–1

wavenumber range. The result is shown on a graph, where X is the wave number, and Y is the percentage of light that gets through.

2.8.3. Identification of Polymer by SEM

The levan surface shape was examined using the scanning electron microscope (SEM Axia Co., Germany) method. Pure freeze-dried levan was stuck to the SEM stubs with double-sided tape, and conductive gold was put on top of it in an ion-sputtering machine. At a speed of 10 kV, the microstructures of samples with different magnifications were looked at. This study obtained SEM images of Levan samples at magnifications of 2500, 5000, 7000, and 13000×.

2.8.4. Identification of Polymer by AFM

The Surface morphology and roughness were obtained using Atomic Force Micrograph (AFM) analysis. First, the levan solution with a 1 mg/ml concentration was prepared and stirred continuously for 1 hour at 40 °C in an airtight bottle. Once cooled to 25 °C, 5µL of levan solution was absorbed into the mica sheet. After levan dried at room temperature, the AFM images were taken by tapping on them.

3. Results and Discussion

3.1. Bacillus spp. Isolation

The isolation process applies selective pressure to obtain bacterial isolates belonging to *Bacillus* spp., known as their ability to produce the levan-forming (levansucrase). *Bacillus* sp. was identified on the agar medium upon their wavy edges, rounded, slightly lobed, irregular shape, creamy, brownish in color, and sticky at times [23]. Morphological tests confirmed the results under microscopic as they possess bacilli or semi-bacilli forms and gram-positive, endospore-forming, with the difference in the spore location according to the isolate species. These characteristics agree with what was mentioned by the taxonomic sources approved and are unique in identifying *Bacillus* bacteria [24].

3.2. Primary Screening

The selection of isolates was performed depending on the high viscosity formed around the colonies in a media containing a high concentration of sucrose as an indication of their ability to produce the enzyme levansucrase, which is the main responsible for levan production. Twenty-six isolates were obtained in this study from different sources according to the abovementioned criteria (Table 1). Eleven of these isolates, which are designated as S-7, S-8, S-9, S-10, S-11, S-12, S-13, S-14, F-3, F-4, and F-5, were distinguished by the amount of sticky material they produced, as well as their high viscosity, similar to the glue around their colonies (colloidal white colonies) as shown in (Figure 1). However, three isolates in the same incubation period gave a very weak viscosity at a rate of 26.92%.

3.3. Secondary Screening

Eleven top levansucrase producers (S-7, S-8, S-9, S-10, S-11, S-12, S-13, S-14, F-3, F-4, and F-5) were subjected to quantitative secondary screening by assay of enzyme activity (U/ml) out of the 26 tested *Bacillus* isolates. It was found that isolate S-8 had a maximum activity estimated at 6.05 U/ml (Figure 2), followed by isolates F4 (4.87 U/ml) and S12 (4.71 U/ml). The minimum activity was found in S11 (2.50 U/ml). After the secondary screening, isolate S-8 was selected for identification at the species level. Several investigators successfully used primary and secondary screening to isolate the microorganism that highly produces levansucrase. As the isolates with the number 8-37-0-1 displayed the highest productivity, Chunhui et al. [25] were able to obtain 42 isolates of bacteria from soil samples taken from Jinan city in China that belong to the genus *Bacillus* and produce the enzyme levansucrase through the formation of levan; the 16S rRNA gene was used to identify the chosen isolate by using information from the GeneBank, it was discovered that it is of the type *Bacillus licheniformis* and has been assigned the registration number KF647836.1. *Bacillus licheniformis* strain RN-01 was discovered by Zhou et al. [26] and identified on the molecular level. It was deposited in GenBank under FJ171619.1 and demonstrated high enzyme productivity when isolated at 50°C from

soil models in the Rinong Province, Thailand, hot springs area. While Phengnoi et al. [27] found that levansucrase-producing bacteria could be isolated from soil samples collected from Maitreya Station in Antarctica, and after conducting the primary and secondary screening processes for the isolates, it was possible to obtain a high-productivity isolate, and after performing the diagnostic process for a gene 16S rRNA and after receiving the mentioned gene sequences and after using the information in the NCBI GeneBank. *B. licheniformis* strain BK2 was isolated from the soil by Permatasari et al. [28], identified using the 16s rRNA gene, and registered in GenBank as MF774878.1

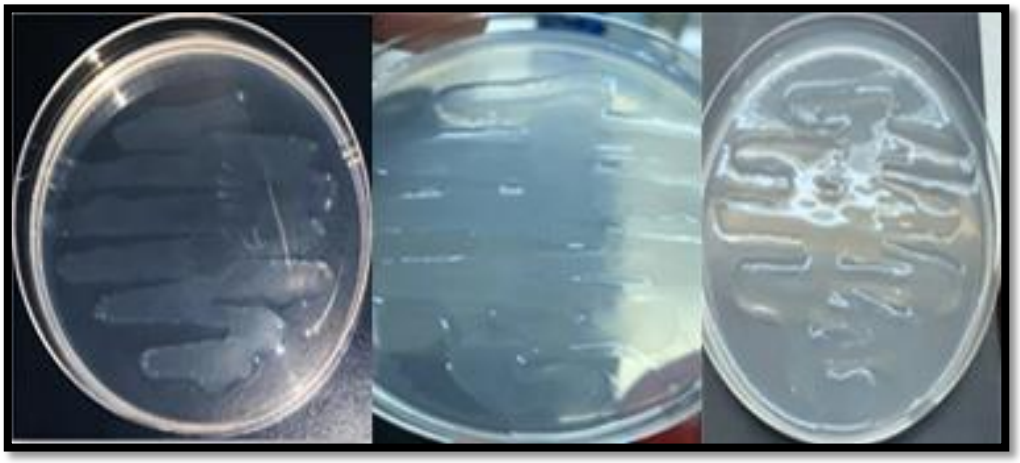


Figure 1. Sticky colonies of different isolates were obtained in the study on the medium of Mineral Salts Agar supplemented with 20% sucrose after incubation for 48 hours.

Table 1. The efficiency of 26 isolates of *Bacillus* sp. to form sticky colonies as an indicator for producing levansucrase in mineral salt agar with 20% sucrose after 48h at 37 C.

Isolates No.	Isolation sources	Samples	Levan forming*
S-1	Amiriya area	Soil of the house garden	++
S-2	Amiriya area	Soil of the house garden	++
S-3	Amiriya area	Soil of the house garden	++
S-4	Amiriya area	Soil of the house garden	++
S-5	Amiriya area	Soil of the house garden	+
S-6	Amiriya area	Soil of the house garden	+
S-7	Fields of the Field Crops Department	Soil of the Potato crop	+++
S-8	Fields of the Field Crops Department	Soil of the Potato crop	+++
S-9	Fields of the horticultural department	Soil of Sativa plant	+++
S-10	Fields of the horticultural department	Soil of Sativa plant	+++
S-11	Fields of the horticultural department	Soil of Sativa plant	+++
S-12	Fields of the horticultural department	Soil of Sativa plant	+++
S-13	Fields of the horticultural department	Soil of Sativa plant	+++
S-14	Fields of the horticultural department	Soil of Sativa plant	+++
C-1	Sweets from vendors' carts	Rotten sweets	+
C-2	Sweets from vendors' carts	Rotten sweets	++
C-3	Sweets from vendors' carts	Rotten sweets	+
C-4	Sweets from vendors' carts	Rotten sweets	+
F-1	Rotten fruit	Persimmon fruit	+
F-2	Rotten fruit	Persimmon fruit	++
F-3	Rotten fruit	Persimmon fruit	+++
F-4	Rotten fruit	Persimmon fruit	+++
F-5	Rotten fruit	Persimmon fruit	+++
J-1	Natural juice residues	Fruit peels	+
J-2	Natural juice residues	Fruit peels	++
A-1	Rotten jam	Carrot jam	++

*+++ High viscosity, ++ Medium viscosity, + Low viscosity.

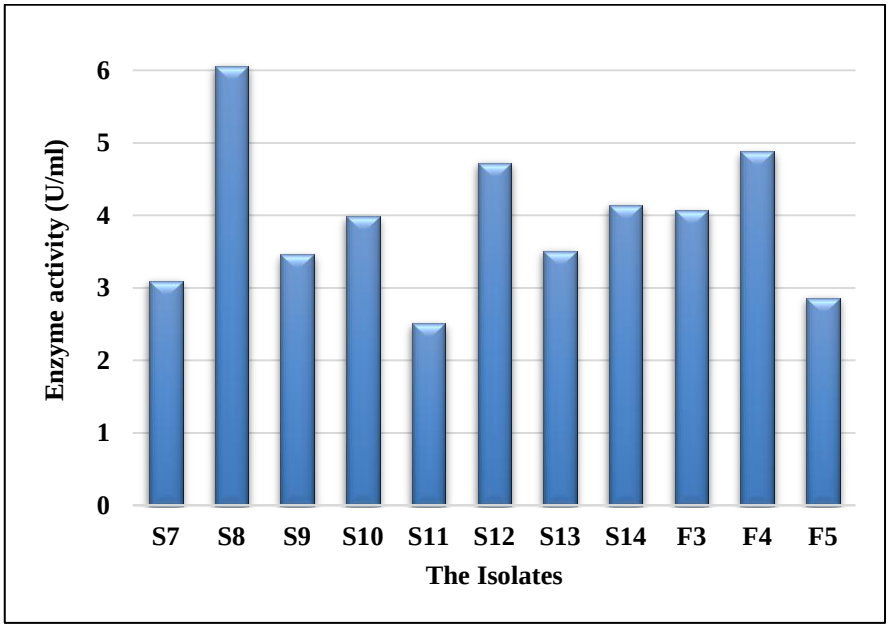


Figure 2. Quantitative secondary screening, the levansucrase activity (U/ml), produced in mineral salt broth medium with 20% at 37°C, 150 rpm, for 48 hours.

3.4. Identification of Selected Isolate S-8

3.4.1. Vitek2 Compact System Identification

The Vitek2 compact system was used to identify the isolate (S-8). The analysis revealed a 92% probability of similarity between this isolate and *Bacillus licheniformis,* as shown in (Figure 3). Neveen et al. [29] pointed out that the adoption of traditional methods in biochemical tests is somewhat complicated due to the need for large quantities of materials, some of which may be expensive, as well as the long time that some tests may take compared to the technology of the Vitek2 system. Funke et al. [30] showed that the Bacillaceae family (BCL) card accurately identifies several aerobic bacteria species forming endospores. Valenza et al. [31] reported a significant difference between the traditional methods of identification results and the results of the Vitek2 system.

Identification Information	Card:	BCL	Lot Number:	2391888103	Expires:	Jan 27, 2023 12:00 CST
	Completed:	Jan 20, 2022 02:40 CST	Status:	Final	Analysis Time:	13.80 hours
Organism Origin	VITEK 2					
Selected Organism	92% Probability		Bacillus licheniformis			
	Bionumber:	1332361755476270			Confidence:	Good identification

Biochemical Details

1	BXYL	+	3	LysA	-	4	AspA	-	5	LeuA	(+)	7	PhcA	+	8	ProA	-
9	BGAL	+	10	PyrA	+	11	AGAL	-	12	AlaA	-	13	TyrA	+	14	BNAG	-
15	APPA	+	18	CDEX	+	19	dGAL	-	21	GLYG	-	22	INO	+	24	MdG	+
25	ELLM	+	26	MdX	-	27	AMAN	-	29	MTE	+	30	GlyA	+	31	dMAN	+
32	dMNE	+	34	dMLZ	-	36	NAG	+	37	PLE	+	39	IRHA	-	41	BGLU	+
43	BMAN	-	44	PHC	-	45	PVATE	(+)	46	AGLU	+	47	dTAG	+	48	dTRE	+
50	INU	-	53	dGLU	+	54	dRIB	+	56	PSCNa	-	58	NaCl 6.5%	+	59	KAN	-
60	OLD	+	61	ESC	+	62	TTZ	+	63	POLYB_R	-						

Figure 3. Results of biochemical tests contained in the Bacillaceae family card (BCL) in the Vitek2 compact system for the most efficient local isolate *Bacillus licheniformis* S-8.

3.4.2. Confirmed Identification of Bacillus Licheniformis

The result of the 16S rRNA gene amplification electrophoresis agarose gel shown on the UV light detector (Figure 4) was a band of 1500 bp. The gene sequence was registered on GenBank under accession number ON81164.1 at strain MJ8. The BLAST nucleotide program was used to compare these sequences with the information in the NCBI [32, 18]. The found isolate was an exact match for the strains of *Bacillus licheniformis* bacteria listed in the NCBI. Furthermore, the study of nitrogenous bases sequence of DNA to identify B. *lichniformans* stated that the 16S rRNA gene had been successfully used to study nitrogenous bases sequence of DNA to distinguish between different types of bacteria, including *Bacillus* spp. specifically, thus, it gives conclusive results in the identification.

This program is adopted when using the maximum probability of finding the phylogenetic tree by the neighbor-joining method and selecting the most accurate form of the possibility of the degree of evolutionary kinship between isolates. Scheme (1) showed an apparent convergence between the local isolate under study and the strains in the NCBI GenBank. It is reported that many researchers have used identification at the molecular level to identify *Bacillus licheniformis* [47].

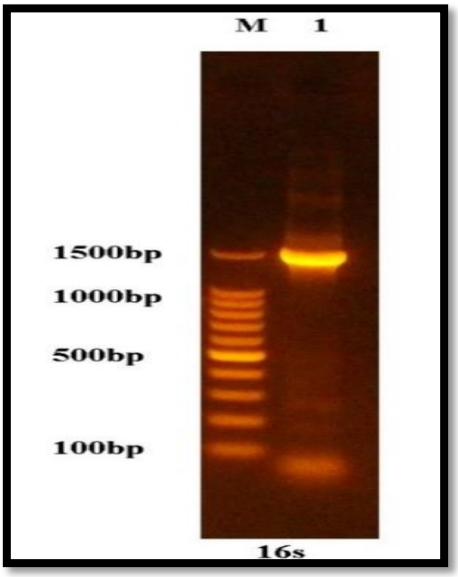
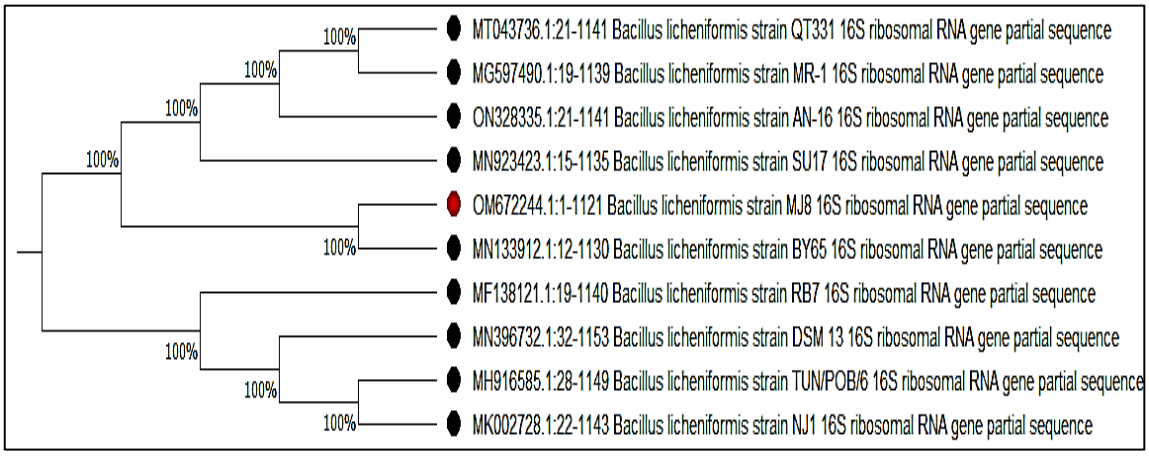


Figure 4. Electrophoresis of PCR product on an agarose gel for 16S rRNA gene for *Bacillus licheniformis* S-8 and marker ladder (100-1500 bp). M: marker, Numbers 1: local isolate



Scheme 1. Phylogenetic analysis of the sequencing identity % of 16S ribosomal RNA gene of NCBI strains with local isolate *Bacillus licheniformis* MJ8 strain.

Note: The analysis was performed with MEGA11 software 11.0.1 by the neighbor-joining method [33].

3.5. The Polymers Identification Structure

3.5.1. HPLC Analysis

To confirm that the polysaccharides from *Bacillus lichniformans* MJ8 in this study belonged to levan, HPLC technology was used to identify them. The retention times for the standard levan from *E. herbicola*, Chicory, and standard of fructose (F), glucose (G), and sucrose (S), in addition to the levan produced in this study, are shown in Figure (5) A, B, C, and D, respectively. The R.T. for the levan under investigation, which was 2.523, 2.533, and 2.540 minutes, coincides with the standard of levan; one peak is observed for the levan under study. Fructose, glucose, and sucrose, on the other hand, had retention times of 2.546, 4.228, and 5.148 minutes, respectively (Table 2). These results show that the levan produced in this study is pure, contains fructose units, and is a homopolysaccharide type [34], in addition to being a fructan type and a member of the fructooligosaccharide (FOS) group. Additionally, Elizabeta et al. [35] successfully produced and separated levan from two isolates, *Zymomonas mobilis* and *E. herbicola*, and analyzed levan using HPLC technology. At the same time, Pei et al. [36] used the HPLC method to demonstrate that the levan structural constituent was 2,6-substituted-fructose.

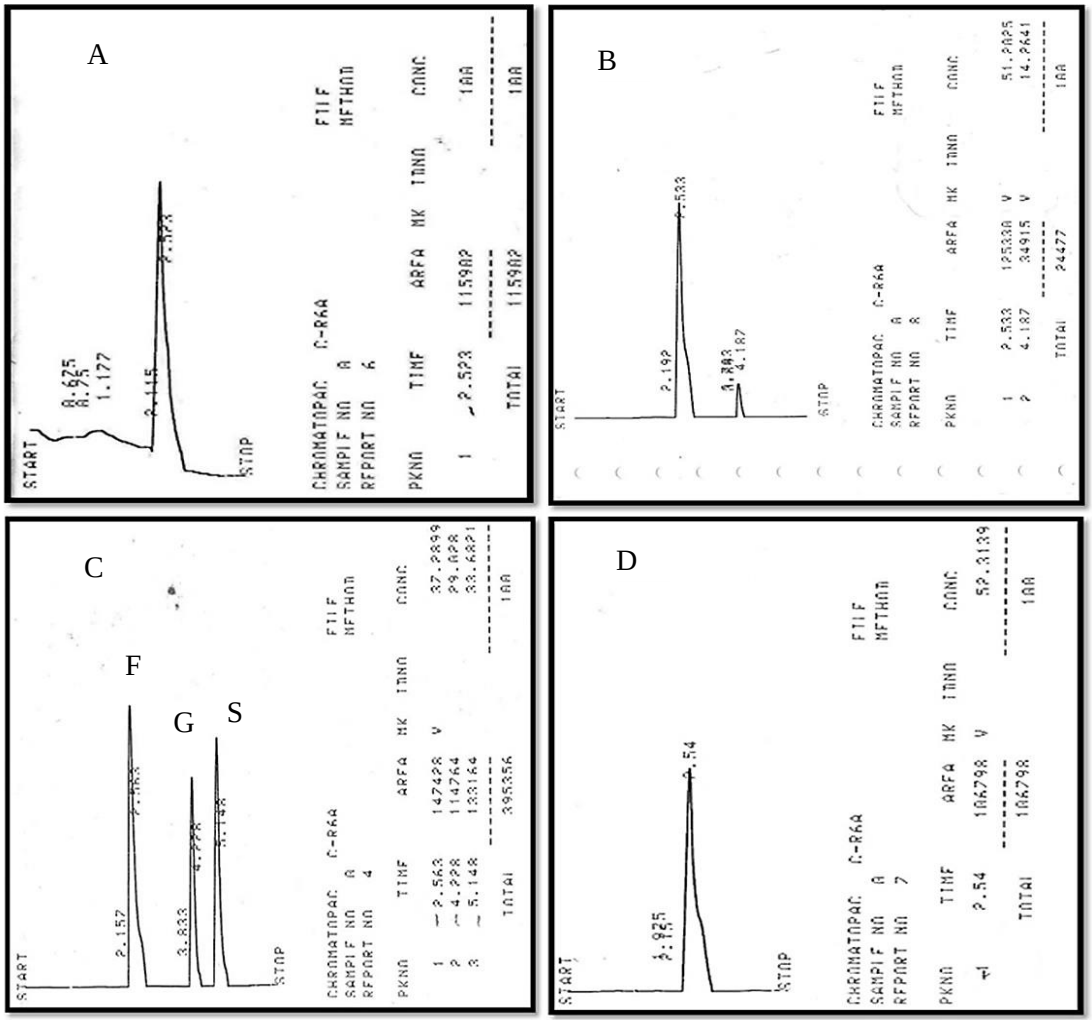


Figure 5. HPLC analysis. A: standard.Levan from *Erwinia herbicola*, B: standard Levan from Chicory. C: sugar solution (F: Fructose, G: Glucose, S: Sucrose),.D: Levan produced in this study from *B. lichniformans* MJ8.

Table 2. The retention time for standard and levan produce from *B. lichniformans* MJ8 using HPLC.

Seq	Samples	Retention time (minute)
A	Standard levan of *E. herbicola*	2.523
B	Standard levan of Chicory	2.533
C-F	Fructose	2.563
C-G	Glucose	4.228
C-S	Sucrose	5.148
D	Levan produced from *B. lichniformans* MJ8	2.540

3.5.2. FTIR Analysis

According to the results of this method, both levan contained the adjustable frequency group C-O at wavelengths of 1123.44 and 1122.07 cm^{-1}, respectively (Figure 6). The bending group, in contrast, begins at CH and OH at wavelengths of 1424.50 and 1425.42 cm^{-1}, respectively, which correspond to the respective frequencies of these groups at 1325.66 cm-1 and 1326.78 cm^{-1}. On the other hand, the ketone groups' stretchable frequencies were found to be 1645.44 cm^{-1} and 1660.14 cm^{-1}, while the C-H groups' adjustable frequencies were found to be 8.58 cm^{-1} and 2933.13 cm^{-1}, respectively, for the two samples (Table 3). The structure of both levan was homologous with the broad stretching peak of O-H stretching at approximately 3319.26cm-1, C-H vibration noted at approximately 2935.48cm^{-1}, and carbonyl C=O spelling recorded at 1722.31cm^{-1}, according to research by Nagnath et al. [37] produced levan from *Pseudomonas fluorescens*. Based on an analysis of the FTIR spectra of levan produced from *B. licheniformis*. Mamay et al. [24] reported that the extension of O-H vibrations first appeared at a wavelength of about 33300 cm-1. At the same time, the peak at wave number 1660cm^{-1} is typical for C=O stretching, while the band around 2900 represents C-H stretching. While Nasir et al. [38] used FTIR to analyze the levan secreted by Halo monas and Chromohalobacter, they discovered that the O-H stretching occurred between 3600 and 3200 cm^{-1}, the C-H stretching occurred between 3000 and 2800 cm^{-1}, the vibration of C=O occurred at 1641.16 cm^{-1}, and the region of typical carbohydrate occurred within the 800-1000 cm^{-1} wavenumber range.

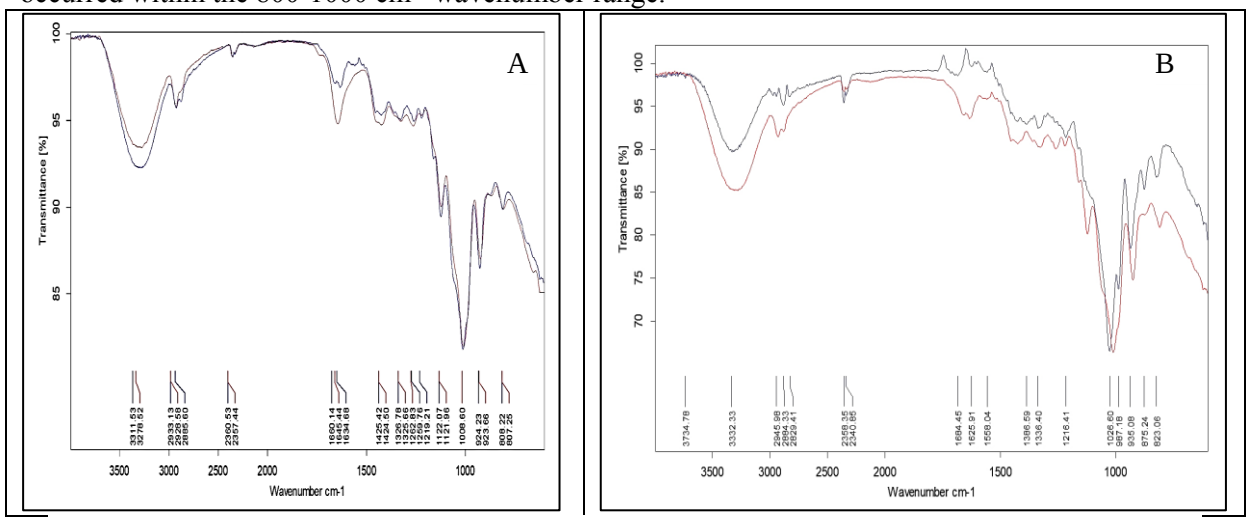


Figure 6. Fourier-transform infrared spectroscopy (A) compares standard levan from *E. herbicola* (Redline) and *B. lichniformans* MJ8 (Blueline), (B) compares standard levan from Chicory (Blueline) and from isolate *B. lichniformans* MJ8 (Redline).

Table 3. The wavenumber peaks (cm^{-1}) of levan produced from *B. lichniformans* MJ8 and compared with standard levan from *E. herbicola* using infrared spectroscopy (FTIR).

No. of Peak	Chemical Groups	Position Wavenumber (cm^{-1})		
		***B. lichniformans* MJ8 Levan**	**Standard Levan of E. herbicola**	**Standard Levan of Chicory**
1	O-H	3278.52	3311.53	3332.33
2	C-H	2928.58	2933.13	2945.98
3		2357.44	2360.53	2358.35
4	C=O	1645.44	1660.14	1684.45

3.5.3. (SEM) Analysis

The levan surface morphology and microstructure were examined using scanning electron microscopy, which can be used to comprehend the levan's physical characteristics better. The surface morphology micrographs of levan at 2500, 5000, 7000, and 13000 (Figure 7). In this study, SEM images showed that Levan had a structure with many branches and many holes. Levan was thought to be most likely to be used as a thickening, stabilizing, and water-binding agent in the food and cosmetics industries because of its highly branched and porous structure, which produces it straightforward for hydrated polymers to form [37]. In addition, SEM images showed that levan had a glossy, sheet-like surface that could be used to create a plasticized film [39]. *Leuconostoc pseudomesenteroides* XG5 produced glucan with a smooth, glittering surface and a highly branched structure. The microstructure of the levan in this study was similar to that of this glucan [40], *Brenneria* sp. and levan from *Bacillus mojavensis* differed little from one another; however, and EniD312 showed a consistent porous network [41, 42].

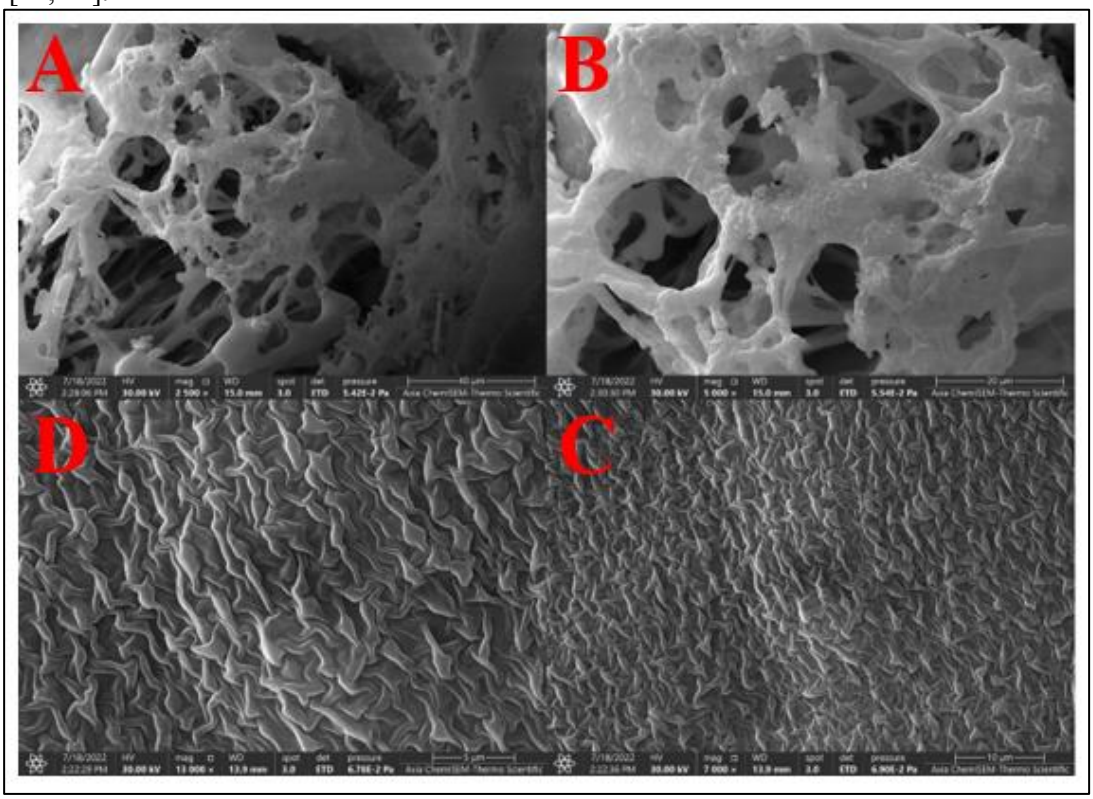


Figure 7. Scanning electron microscope image of levan produced in this study, where A: 2500X, B: 5000X, C: 7000X, D: 13000X.

3.5.4. (AFM) Analysis

AFM, created based on SEM, is a valuable tool for characterizing polymer morphology with high resolution and ease of use. Levan topographical AFM images showed numerous ellipsoidal or

spheroidal particles and lumps resembling spikes, demonstrating that oligosaccharides had a strong affinity for the water molecules [43, 44]. The conclusion depicted in Figure (8) indicates that levan intermolecular and intramolecular aggregation may cause the tightly packed molecular structure seen in AFM images [34]. The polymer from *Lactobacillus sake* 3 was reported to have produced similar results [42] but was distinct from the tangled networks of *Lactobacillus reuteri* E81 glucan [45], and Mesona blames gum EPS polymer, which was shaped erratically like a worm [46].

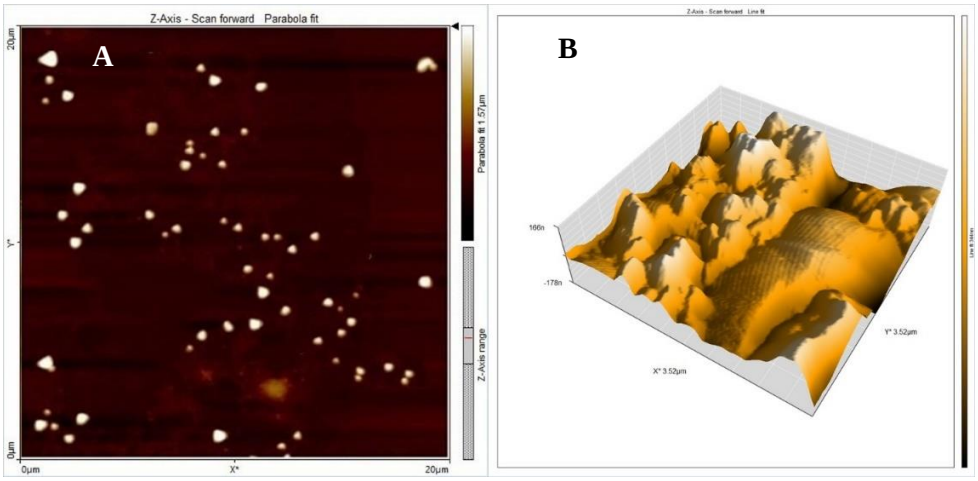


Figure 8. images of AFM for purified levan (A) planar, (B) 3D.

Conclusion

A novel strain of *Bacillus* sp. that can produce levansucrase was isolated from the soil rhizosphere of the Stevia plant. The identification tests with the Vitek2 system and at the molecular level confirmed that this local isolate belongs to *B. lichniformans and* was called *B. lichniformans* MJ8. The identification results of levan using HPLC technology showed the presence of fructose when acidic decomposition. The possibility depends on one of the characterization techniques to identify levan. The best technique for levan characterization was shown to be the FTIR technique. The structure of the synthetic levan and that of natural levan were remarkably similar. Before examining potential applications, future research will concentrate on the biological role of the enzymatically produced levan.

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Comparison of Different Types of Feed Additives (Premix) and their Effect on Production Performance for Broiler Ross 308

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Abstract. This study was conducted in the farm, of the poultry research station of the animal resources Research Department / Office of Agricultural Research / Ministry of Agriculture, for the period from 2/7/2022 to 5/8/2022 for 35 days , and the experiment aimed to know the comparison of different types of feed additives (premix) And its impact on the productive performance of Ross 308 broilers, as 900 Ross 308 broiler chicks were used at one day old and were randomly distributed to three treatments, each with ten replicates, thirty chicks per replicate (300 chicks per treatment) and the treatments were: The first treatment (T1) was the use of protein concentrate at a rate of 5%, the second treatment was the use of a Formix feed mixture of the type of DSM company at a rate of 2.5%, and the third treatment was the use of a commercial forage mixture at a rate of 2.5%, the results showed a significant superiority ($P<0.05$) in the rate of The live body weight for the fifth week and the overall weight gain rate for the second treatment compared to the other treatments, and there were no significant differences between the treatments in the amount of feed consumed ,and there was a significant improvement for the second treatment compared to the first and third treatments, and there was no significant difference between the first and third treatments for the periods 11-24 and 1-35 days in the feed conversion factor.

Keywords. Feed additives, Premix and Formix, Poultry farmer.

1. Introduction

Poultry farmer seek to obtain the best results in terms of production value and at the lowest financial costs to make the most of the available feed materials [1]. Production and reproduction, through the manufacture of balanced diets, and according to the above, nutrition is the main factor on which the production process depends [2] . For decades, the poultry industry has relied on the use of protein concentrates of animal origin and then of plant origin, then main sources in the preparation of bird feeds with amino acids, protein, vitamins, minerals and some other feed additives with specific goals [3,4]. Therefore, global feed manufacturing companies worked with feed support materials called premixes, which are a mixture of amino acids, vitamins, minerals and other materials such as enzymes and antioxidants with a carrier substance, which in turn is mixed in the composition of the feed [5]. Also, these feed mixtures or premixes differ in their content of amino acids, vitamins, minerals and

other additives, and each commercial product differs from the other in providing the bird with its needs, as well as in the food conversion factor, feed consumption and weight gain [6]. One of the important benefits for specialists in feeding poultry birds when using premix, as it is one of the important clues to knowing the components of the diet of vitamins, amino acids and minerals, and thus obtaining balanced diets that meet all the needs of the bird, in addition to reducing some nutritional diseases that occur as a result of a deficiency in one Nutrients [7]. The addition of premixes significantly improves body weight and reduces stress on chicks [8]. This study aims to compare different types of feed additives (premix) and their effect on the productive performance of Ross 308 broilers.

2. Materials and Methods

This study was conducted in the poultry research fields of the Agricultural Research Department / Ministry of Agriculture and Agriculture from 2/7/2022 to 5/8/2022 for a period of 35 days, the study aimed to know the different types of feed additives (premix) and their effect on production performance for broiler Ross 308 , as 900 unsexed Ross 308 broiler chicks were used, with an average weight of 35.5 gm at the age of one day, distributed randomly into three treatments, each with ten replicates, with thirty chicks for each replicate (300 chicks for each treatment) and the treatments were distributed as follows: The first treatment (T1) was Add protein concentrate by 5%, and the second treatment (T2) was added to a wheat based Formix feed mixture. From the DSM company by 2.5% (and its diet was formed according to the recommendations of the company that produced the premix from corn, soy, vegetable oil and limestone in addition to premix), as for the third treatment (T3), a commercial feed mixture was added at a rate of 2.5%, the chicks were fed on a starter ration From the age of (1-10) days and on the growth ration at the age of (11-24) days and the final ration at the age of (25-35) days according to the Ross 308 guide for the year 2019 as shown in Table (1) The preventive program followed in the animal field of the poultry research station was used, and the following productive traits were studied: live body weight, weight gain, consumed feed and feed conversion factor. Completely randomized design (CRD) was used to study the effect of treatments on the studied traits. The significant differences between the means were compared with Duncan's polynomial test [9], and the statistical program SAS - Statistical Analysis System [10] was used in the statistical analysis of the data.

Table 1. The calculated percentages and chemical composition of the broiler diet Ross 308.

Feed material %	Starter 1-10 days			Grower 11-24 days			Finisher 25-35 days		
	First treatment (T1)	Second treatment (T2)	Third treatment (T3)	First treatment (T1)	Second treatment (T2)	Third treatment (T3)	First treatment (T1)	Second treatment (T2)	Third treatment (T3)
Yellow corn	58.4	60	58.9	60.17	63	61.78	64.18	66	64.85
Soybean meal 48%	33.4	35	36.6	30	31.5	33	25.2	28	28.4
Vegetable oil	1.4	1	0.5	3	1.5	1.6	4	2	3.1
Limestone	1.8	1.5	1.5	1.7	1.5	1.12	1.5	1.5	1.1
Lysine	——	——	——	0.13	——	——	0.12	——	
Protein Concentrate 5% *	5	——	——	5	——	——	5	——	——
Premix Formex 2.5%	——	2.5	——	——	2.5	——	——	2.5	——
Commercial premix 2.5%	——	——	2.5	——	——	2.5	——	——	2.5
Total	100	100	100	100	100	100	100	100	100

	Calculated Chemical Analysis **								
	First treatment (T1)	**Second treatment (T2)**	**Third treatment (T3)**	**First treatment (T1)**	**Second treatment (T2)**	**Third treatment (T3)**	**First treatment (T1)**	**Second treatment (T2)**	**Third treatment (T3)**
Total energy	3002	2976	3003	3107	3028	3100	3204	2967	3210
Crude protein	22.99	22.59	22.99	21.51	21.10	21.50	19.55	22.39	19.56
Calcium	0.93	0.83	0.96	0.89	0.88	0.82	0.80	0.96	0.80
Lysine	1.27	1.37	1.34	1.29	1.26	1.25	1.16	1.29	1.17
Methionine	0.54	0.69	0.57	0.52	0.62	0.55	0.50	0.57	0.53
Methionine + Cysteine	0.89	1.03	0.92	0.85	0.94	0.88	0.80	0.91	0.83
Threonine	0.89	0.97	0.89	0.83	0.89	0.83	0.75	0.92	0.75

* Annex (1) Chemical analysis of each of the protein concentrate and premix type Formex and commercial premix.

** The values of chemical analysis of the feed materials included in the diet were calculated according to [11] .

3. Results

3.1. Live Body Weight

We note from Table (2) that there were no significant differences between the experimental treatments in live body weight at the age of 10 days. At the age of 24 and 35 days, the second treatment was significantly (P<0.05) outperformed compared to the first and third treatments, which had no significant differences. between them.

Table 2. Comparison of different types of feed additives (premix) and their effect on average live body weight (gm/bird) for Ross 308 broilers (means ± standard error).

Age (day)/ **Treatments	**10 -1**	**24 -11**	**35 – 25**
First treatment (T1)	4.954 ± 214.02	b* 19.524 ± 922.37	b 26.488 ± 1906.91
Second treatment (T2)	9.855 ± 218.69	a 18.811 ± 1011.38	a 21.384 ± 2018.98
Third treatment (T3)	7.806 ± 230.52	b 10.988 ± 950.57	b 14.234 ± 1927.36

*Different letters within the same column indicate significant differences at the (P<0.05) probability level.

** The first treatment (T1) was using protein concentrate by 5%, the second treatment (T2) using a Formix feed mixture from DSM company at a rate of 2.5%, and the third treatment (T3) using a commercial forage mixture at a rate of 2.5%.

3.2. Weight Gain Rate

Table (3) shows a comparison of different types of feed additives (premix) and their effect on the rate of weight gain of Ross 308 broilers, to the absence of a significant difference between treatments during the period from 1-10 days, while a significant superiority (P<0.05) is observed when The age of 11-24 days for the second treatment over the first and third treatments, and there was no significant difference between the first and third treatments, and we note from the same table that there is no significant difference between all the experimental treatments in the period from 25-35 days of age, as it is noted from In the table, there was a significant superiority (P<0.05) for the total cumulative period from 1-35 days in favor of the second treatment compared to the first and third treatments, which had no significant difference between them.

Table 3. Comparison of different types of feed additives (premix) and their effect on the rate of weight gain (gm/bird) for Ross 308 broilers (means ± standard error).

Age (day)/ **Treatments	10 -1	24 -11	35 – 25	1 – 35
First treatment (T1)	5.007 ± 177.97	b* 16.956 ± 708.35	13.701 ± 984.55	b 26.518 ± 1870.86
Second treatment (T2)	9.834 ± 182.69	a 10.914 ± 792.69	18.957 ± 1007.60	a 21.450 ± 1982.00
Third treatment (T3)	7.775 ± 194.62	b 9.677 ± 720.05	12.959 ± 976.80	b 14.214 ± 1891.46

*Different letters within the same column indicate significant differences at the (P<0.05) probability level.
** The first treatment (T1) was using protein concentrate by 5%, the second treatment (T2) using a Formix feed mixture from DSM company at a rate of 2.5%, and the third treatment (T3) using a commercial forage mixture at a rate of 2.5%.

3.3. Feed Consumption Rate

The results of the statistical analysis in Table (4) indicate that there are no significant differences for all treatments in the rate of feed consumption during the age periods from 1-10, 11-24, 25-35 days, and the total from 1-35 days.

Table 4. Comparison of different types of feed additives (premix) and their effect on feed consumption rate (gm/bird) for Ross 308 broilers (means ± standard error).

Age (day)/ **Treatments	10 -1	24 -11	35 – 25	1 – 35
First treatment (T1)	4.396 ± 251.00	33.102 ± 988.79	18.030 ± 1513.90	52.146 ± 2753.70
Second treatment (T2)	5861 ± 252.61	23.968 ± 987.02	15.298 ± 1534.13	38.447 ± 2773.76
Third treatment (T3)	5.985 ± 252.54	27.581 ± 1009.13	15.292 ± 1546.45	30.279 ± 2808.12

*Different letters within the same column indicate significant differences at the (P<0.05) probability level.
** The first treatment (T1) was using protein concentrate by 5%, the second treatment (T2) using a Formix feed mixture from DSM company at a rate of 2.5%, and the third treatment (T3) using a commercial forage mixture at a rate of 2.5%.

3.4. Feed Conversion Factor

With regard to the feed conversion factor, Table (5) shows that there were no significant differences between the treatments in the feed conversion factor for the two periods from 1-10 and 25-35 days, while at the age of 11-24 and 1-35 days it improved significantly (P<0.05). The second treatment compared with the first and third treatments, and there was no significant difference between the first and third treatments.

Table 5. Comparison of different types of feed additives (premix) and their effect on the feed conversion factor (gm/bird) for Ross 308 broilers (means ± standard error).

Age (day)/ **Treatments	10 -1	24 -11	35 – 25	1 – 35
First treatment (T1)	0.026 ± 1.41	a* 0.032 ± 1.39	0.024 ± 1.53	a 0.015 ± 1.47
Second treatment (T2)	0.054 ± 1.40	b 0.02 ± 1.24	0.027 ± 1.52	b 0.016 ± 1.39
Third treatment (T3)	0.02 ± 1.30	a 0.048 ± 1.40	0.023 ± 1.58	a 0.018 ± 1.48

*Different letters within the same column indicate significant differences at the (P<0.05) probability level.
** The first treatment (T1) was using protein concentrate by 5%, the second treatment (T2) using a Formix feed mixture from DSM company at a rate of 2.5%, and the third treatment (T3) using a commercial forage mixture at a rate of 2.5%.

4. Discussion

The reason for the moral superiority that occurred in the second treatment may be due to the premix containing amino acids, vitamins, minerals and some digestive and metabolic enzymes, which led to the body benefiting from these substances and thus reflected positively on body weight [12]. This may be attributed to The superiority of the premix treatment of Formex in the second treatment to the fact that the chicks in the first days are exposed to stress as a result of shifting nutrition after they were on the yolk sac, which is characterized by its high content of proteins and fats and the lack of

carbohydrates, so the process of filling fat and lipoproteins decreases in order to save energy and operations Vitality in the body and usually accompanied by a decrease in the liver content of vitamin E [5]. And the provision of high levels of vitamins in this period reduces the stress that was available in the mixtures used and made them available in a high way in the type of Formex compared to other mixtures, also that the premix type of Formix contains multiple enzymes in addition to the enzyme phytase, which is not available in other mixtures As these enzymes work to break down and replace the components of the cell walls of cereal grains consisting of the diet containing non-starch polysaccharides, reduce the viscosity of the gastrointestinal tract, and increase the level of digestible protein, lysine and energy metabolism in the ileal region [13], and that Protein and amino acid digestion improves with increased consumption of multienzyme food [14].

5. Annex (1): Components of the Feed Mixtures Used in the Experiment

5.1. Protein Concentrate (5% addition)

Each kilogram of protein concentrate contains a total energy of 2390 kcal, crude protein 40%, crude fat 2.40%, crude fiber 4.40%, sodium 3%, lysine 4.5%, methionine 4.6%, methionine + cysteine 5.1%, threonine 2% and phosphorous Total 2.9%, phosphorous available 5.5%, vitamin A 200000IU and vitamin D3 60000IU, and a group of mineral elements, antioxidants, phytase enzyme, anti-mold and fish oil were also added.

5.2. Formix is a Type of Formix Manufactured by DSM Company

5.2.1. First Stage (Starter)

One kilogram equals to protein 27.63%, raw 1314.50 kcal/kg calcium 7.79%, phosphorous 8.07%, lysine 109200 mg/kg, methionine 148000 mg/kg threonine 58101 mg/kg and citric acid 50 oxidation is BHT 331 mg Propyl gallate 28 mg/kg sepiolite 20000 mg/kg phytase 80000 FYT/g beta-glucanase 0.300 FYT/g beta-cyanides 8000 mg/kg iron 800 mg/kg copper 640 mg/kg manganese 4800 mg / kg and Zinc is 4400 mg/kg, iodine 50 mg/kg, selenium is 12015 µg/kg, vitamins are A 480000 IU/kg, D3 is 200000 IU/kg, E is 3200 mg/kg, K3 is 128 mg/kg, B1 is 128 mg/kg, B2 344 mg/kg calcium, D-pantothene 800 mg/kg, B6 172 mg/kg, B12 780 mcg/kg, niacin 2600 mg/kg, folic acid 88 mg/kg, biotin 8800 mcg/kg, choline chloride 22400 mg/kg.

5.2.2. Second Stage (Grower Growth)

One kilogram contains 25.009% crude protein, a representative energy of 1177 kcal/kg, calcium 10.19%, phosphorous 7.17%, lysine 102960 mg/kg, methionine 148126 mg/kg, threonine 51221 mg/kg, citric acid 50 mg/kg and antioxidants. They are Butylated hydroxytoluene 331 mg/kg, Propyl Gallate 28 mg/kg, sepiolite 20000 mg/kg, phytase 80000 FYT/g, butylated hydroxytoluene 0.300 FYT/g, beta-xylanase 8000 mg/kg, and the minerals are iron 800 mg/kg and copper 640 mg/kg, manganese 4800 mg/kg, zinc 4400 mg/kg, iodine 50 mg/kg, selenium 12015 µg/kg, vitamins A 400000 IU/kg, D3180000 IU/kg, E 3600 mg/kg, K3 100 mg/kg, B1100 mg/kg, B2 260 mg/kg, calcium, D-pantothene 120 mg/kg, B6 128 mg/kg, B12 680 µg/kg, niacin 2,400 mg/kg, folic acid 76 mg/kg, Biotin 7200 mcg/kg and choline chloride 22400 mg/kg.

5.2.3. Third Stage (Finisher)

One kilogram contains 19.855% crude protein, a representative energy of 933.995 kcal/kg, calcium 12.68%, phosphorous 7.17%, lysine 79560 mg/kg, methionine 100,800 mg/kg, threonine 39401 mg/kg, citric acid 50 mg/kg, and antioxidants. The oxidants are Butylated hydroxytoluene 331 mg/kg, Propyl Gallate 28 mg/kg, sepiolite 20000 mg/kg, phytase 80000 FYT/g, butylated hydroxytoluene 0.300 FYT/g, beta-xylanase 8000 mg/kg, and the mineral elements are iron 800 mg/kg and Copper 640 mg/kg, manganese 4800 mg/kg, zinc 4400 mg/kg, iodine 50 mg/kg, selenium 12016 µg/kg, vitamins A 360000IU/kg, D3 160000 IU/kg, E 2200 mg/kg, K3 88 mg/kg, B188 mg/kg, B2 216 mg/kg, calcium, D 600, biotin 6000 mcg/kg, B6 88 mg/kg, B12 440 mcg/kg, niacin 1800 mg/kg, folic acid 64 mg / kg and choline chloride 22400 mg / kg.

5.3. Commercial Premix: (the Percentage of Addition is 2.5%)

5.3.1. First Stage (Starter)

Each one kilogram contains crude protein 16.7%, total energy 3897 kcal, lysine 8.1%, methionine 9.6%, methionine + cysteine 9.6%, threonine 1.5%, calcium 12.4%, phosphorus 7.2%, sodium 6.4% and all vitamins The following are: A 480000 IU, D3 140000 IU, E 2000 IU, K 120 ppm, B1 160 ppm, B2 300 ppm, calcium D-pantothenate 652 ppm, B3 2000ppm, B7 6000 ppb, B12 1000 ppb, B9 40 ppm, B6 200 ppm and add From betaine, as well as each of the following mineral elements: iron 2800 ppm, copper 600 ppm, zinc 2400 ppm, magnesium 2800 ppm, iodine 80 ppm, selenium 10 ppm, and also the addition of phytase enzyme, salinomycin - sodium, xylanase, propyl gallate and butylatehhydroxytoluene.

5.3.2. Second and Third Stage (Grower and Finisher)

One kilogram contains 15.2% crude protein, a total energy of 3820 kcal, lysine 7.2%, methionine 9%, methionine + cysteine 9%, threonine 1.7%, calcium 17.8%, phosphorous 3.3%, sodium 6.1% and the following group of vitamins: A 400000 IU, D3 100000 IU, E 1600 IU, K 80 ppm, B1 80 ppm, B2 300 ppm, calcium D-pantothenate 435 ppm, B3 1400 ppm, B7 4000 ppb, B12 1000 ppb, B9 40 ppm, B6120 ppm, C 4000 ppm Add From betaine, as well as each of the following mineral elements: iron 2800 ppm, copper 600 ppm, zinc 2400 ppm, magnesium 2800 ppm, iodine 80 ppm, selenium 10 ppm, and also adding phytase enzyme, salinomycin - sodium, xylanase, propyl gallate, butylatehydroxytoluene.

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Identification and Determination of Amino Acids in Leaf and Whole Fruit Powder of Neem (*Azadirachta indica*)

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Abstract. This study aimed to diagnosis and determine of amino acids in the powder of whole neem leaves and fruits, after the collection, washing, drying and grinding process, the results of our study showed that chemical detection of amino acids in the powder of neem leaves and fruits contain (Aspartic acid, Glutamic acid, Serine, Histidine, Glycine, Threonine Arginine, Alanine, Tyrosine, Cystine, Valine, Methionine, Phenylalanine, Isoleucine, Leucine, Lysine), whereas their concentration in fruit powder was (0.1, 24.0, 5.7, 4, 4.6, 4.6, 0.9, 25.0, 3.0, 3.5, 3.1, 6.3, 0.8, 3.5, 14.9, 0.9, 0.9) %, respectively. While their concentration was In leaf powder (5.0, 22.9, 7.3, 0.1, 15.0, 0.1, 13.2, 1.2, 1.6, 2.9, 8.0, 0.4, 4.4, 3.0, 0.7, 0.2)%, respectively. The conclusion from this study are the powder of neem leaves and fruits contains a good proportion of essential and non-essential amino acids.

Keywords. *Azadirachta indica*, Amino acid, Chromatography analyzer of amino acids, Phytonutrients.

1. Introduction

The neem plant is scientifically referred to as (*Azadirachta indica*) and has several names, including (Indian lilac), which is an evergreen tropical and subtropical plant, belongs to the family Meliaceae, and this plant thrives in climates with an annual rainfall of 400-800 mm and a dry season It is a fast-growing and evergreen tree with a height of 15-30 meters. The vegetative are growth characterized by long leaves (20-40 cm), alternate, pinnate, excessive, light green in color, each consisting of 8-19 leaves. Serrated leaflets alternate closely, as the neem plant is one of the woody trees that is distinguished by a large, deep root system that extends over a wide area and horizontally. The flower of the neem plant is white or pale yellow in color and smells sweet, while its fruit is single and small in size (1-2 cm) green in color and turns yellow when ripe. The neem fruit contains a brown seed that is oval or spherical [1,2]. In the Indian subcontinent, the neem tree has been used for more than 4,500 years, where it is mainly cultivated there. The origin of the neem tree is from the regions of Pakistan, India, Bangladesh and Myanmar. The neem tree was introduced to places such as Australia, east and south of the African coast, southeast Asia and South America, and humanity used it on A wide range of treatment for various diseases anciently till now. Furthermore, all the parts of this tree are usually used in traditional Indian medicine to treat various diseases [3].

2. Materials and Methods

2.1. Collecting and Preparing Samples of Neem Fruits and Leaves

The plant and fruits materials of the neem plant were collected during September of the year 2021 from the orchard of an agricultural engineer in the Rawa district of Anbar Governorate in Iraq. The fruits and leaves were soaked in tap water for (2-5) minutes to remove the dust and impurities, then washed twice with distilled water. After that, it was spread on polyethylene leaves in the shade and at a room temperature (25) °C, with proper ventilation and stirring from time to time to speed up the drying process and prevent the growth of mold on it, which lasted for (2-3) weeks until the leaves and fruits were completely dry. After that, the whole leaves and fruits were ground (pulp and seed) using an electric grinder, and they were packed in polyethylene bags in order not to be exposed to moisture and pollution and kept in the freezer till the study performed.

2.2. Preparation of a Sample of Leaves and Fruits of Neem

The samples of leave and fruit for examination was followed by [4], as 3 g of powdered leaves or fruits under study were taken and digested with 25 ml of hydrochloric acid $HC_{l6}N$ in an electric oven at a temperature of 110 C for 24 hours, then the mixture was filtered using filter paper (Whattman No. 8). Accordingly, get rid of the hydrochloric acid by a rotary evaporator at a temperature of 40 °C, after which the resulting dry part was dissolved in a buffer solution of 0.2 N sodium citrate (pH 2.2) to make the sample ready for injection into the amino acid analyzer.

2.3. Analysis of Leaf and Fruit Powder and Separation of Amino Acids Using an Amino Acid Analyzer Chromatography

A sample of the leaves and fruits of the neem plant was analyzed and the amino acids were separated using a chromatography device, an Amino Acid Analyzer, type sykam of German origin, by adopting the separation conditions used by [5], where a column filled with cation exchange resin and a carrier phase consisting of (a reagent) was used. OPA, Buffer buffer) and the injection volume of the sample was between 1-5000 µL and a pressure of 150-24 bar, while the temperature of the column used was between 20-99 °C, and amino acids were detected using a photometer at a wavelength ranging between 570-440 nm.

2.4. Diagnosis and Determination of Amino Acids

Amino acids were diagnosed and estimated in the powder of leaves and fruits of the neem plant, which appeared on the computer of the Amino acid analyzer device, by comparing it with the retention time for a number of standard amino acids as shown in Table (1), as the standard solution contained 16 amino acids, where it was treated as same as the whole treatments and conditions in which the study samples were implemented.

Table 1. Amino acids were diagnosed and estimated in the powder of leaves and fruits of the neem plant, coupled with the retention time.

No.	Amino acid	Retention time (Minute)	No.	Amino acid	Retention time (Minute)
1	Aspartic	8.908	9	Tyrosine	13.868
2	Glutamic	10.756	10	Cystine	16.024
3	Serine	11.824	11	Valine	17.212
4	Histidine	12.196	12	Methionine	17.568
5	Glycine	12.368	13	Phenylalanine	18.072
6	Threonine	12.716	14	Isoleucine	18.460
7	Arginine	13.276	15	Leucine	18.788
8	Alanine	13.348	16	Lysine	18.972

3. Results and Discussion

3.1. Detection of Amino Acids in Leaf and Fruit Powder of Neem Plant Amino Acid Content of Neem Fruits

The process of analyzing a sample of neem powder by means of the chromatography Amino Acid Analyzer resulted in the detection and identification of sixteen amino acids by drawing a standard curve and a peak for each acid coupled with its retention time, which is illustrated by the chromatography diagram in Figure (1), and as evidenced by This chart shows that there are some other amino acids separated that were not diagnosed by the device because there are no standard compounds for them, while Table (2) shows the type and concentration of the diagnosed amino acids, as a discrepancy appears in the concentrations of these acids present in the fruits of neem, so the ratio was the highest level is for the amino acid arginine, which was found at a rate of 25%, followed by the amino acid glutamic acid at a rate of 24%, while we did not stand on a study in which the content of whole neem fruits was estimated from amino acids, but it was mentioned [6] when studying the composition of amino acids in Neem seeds, the highest percentage was for glutamic acid, which was found at a rate of 23.65%, followed by aspartic acid, at a rate of 9.62%, as the ratio of these two acids was close to their ratio in our study.

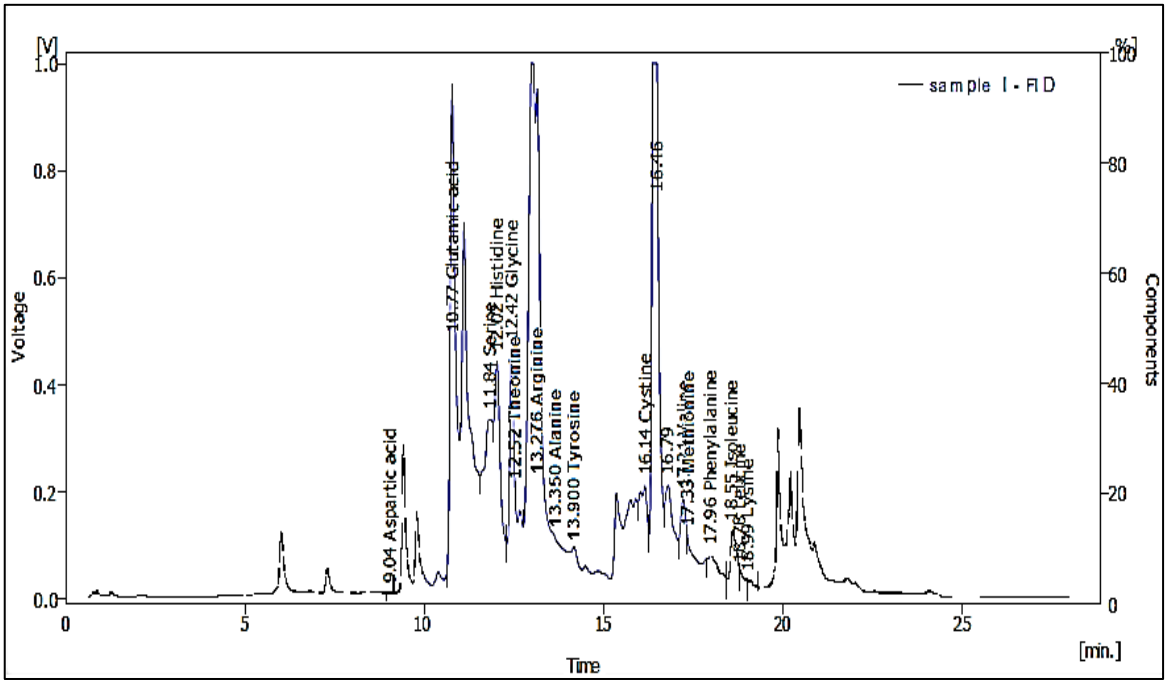

Figure 1. Standard curve and a peak for each acid coupled with its retention time in the fruits of neem plant.

Table 2. The type , coupled with the retention time and concentration of the diagnosed amino acids in the fruits of neem plant.

No.	Amino acid	Retention time (Minute)	Concentration (%)
1	Aspartic	9.036	0.1
2	Glutamic	10.768	24.0
3	Serine	11.840	5.7
4	Histidine	12.020	4.0
5	Glycine	12.424	4.6
6	Threonine	12.524	0.9
7	Arginine	13.276	25.0
8	Alanine	13.350	3.0

No.	Amino acid	Retention time (Minute)	Concentration (%)
9	Tyrosine	13.900	3.5
10	Cystine	16.144	3.1
11	Valine	17.212	6.3
12	Methionine	17.332	0.8
13	Phenylalanine	17.956	3.5
14	Isoleucine	18.548	14.9
15	Leucine	18.780	0.9
16	Lysine	18.992	0.9

3.2. The Content of Neem Leaves of Amino Acids

The result obtained from the analysis of neem leaves powder under study by the chromatography amino acid analyzer revealed sixteen amino acids, which were shown by drawing the peaks and standard curves of these acids, whereas associated with the retention time for each of them, as shown by the chromatography diagram in Figure (2). Also, it is clear from the chart that some peaks and curves appear for other amino acids separated, but they have not been diagnosed and recognized by the device due to the lack of standard compounds for them, while Table (3) shows the type and concentration of the diagnosed amino acids, as there was a difference in The concentrations of these acids, as neem leaves were characterized by a high proportion of the amino acid glutamic acid, which was found at a rate of 22.9%, followed by the amino acid glycine at a rate of 15%. And the percentage of the highest value was for the amino acid glutamic acid, which amounted to 22.56 grams / per 100 grams of protein.

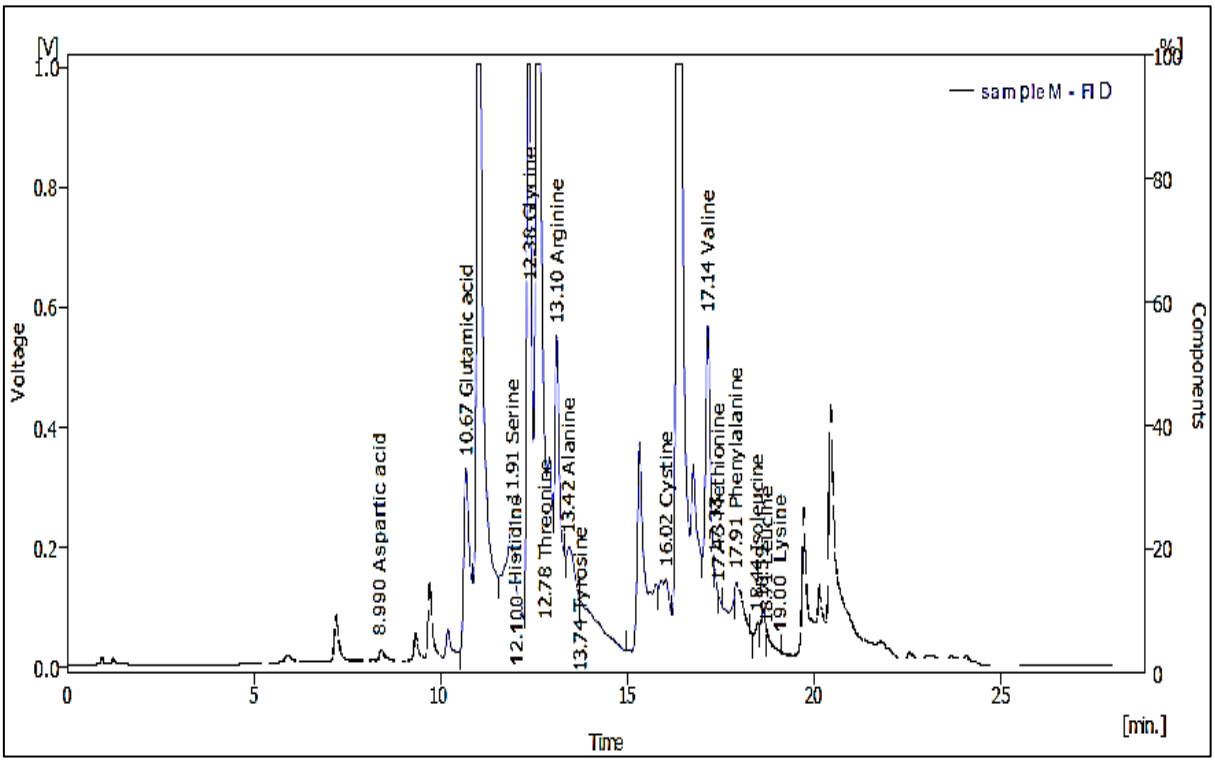

Figure 2. Standard curve and a peak for each acid coupled with its retention time in the neem leaves powder.

Table 3. The type, coupled with the retention time and concentration of the diagnosed amino acids in the in the neem leaves powder.

No.	Amino acid	Retention time (Minute)	Concentration (%)
1	Aspartic	8.990	5.0
2	Glutamic	10.668	22.9
3	Serine	11.912	7.3
4	Histidine	12.100	0.1
5	Glycine	12.380	15.0
6	Threonine	12.780	0.1
7	Arginine	13.096	13.2
8	Alanine	13.420	1.2
9	Tyrosine	13.736	1.6
10	Cystine	16.020	2.9
11	Valine	17.144	8.0
12	Methionine	17.426	0.4
13	Phenylalanine	17.908	4.4
14	Isoleucine	18.444	3.0
15	Leucine	18.712	0.7
16	Lysine	19.00	0.2

Conclusions

The fact that the fruits and leaves of the neem plant contain a high percentage of the amino acid glutamic, which is used in the biosynthesis of proteins by almost all living organisms and plays a crucial role in the process of cellular metabolism, in addition to the fact that the leaves and fruits contain a good percentage of other essential and non-essential amino acids makes this plant is one of the important food sources that can provide the human body with what it needs of these acids, which are classified as macronutrients, and the fruits of neem contain a high percentage of the amino acid arginine, which is one of the basic amino acids that humans need because of its inability to manufacture it. It makes this fruit an important food source in filling the body's need for this acid.

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Studying of Soft Cheese Manufacturing using New Techniques to Improve its Shelf Life and Quality Properties

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Abstract. The purpose of this study was to acertain the impact of utilizing various starters on the physico-chemical, sensory, and rheological characteristics of soft cheese made from fresh full-fat cow's milk. The first treatment involved adding a mixture of skim, dried milk, and vegetable fat and starter by treatment (T1, T2, and T3) in a certain ratio to the control treatment (C) . All cheeses were stored at a temperature of (4 $\pm$1) C For a period of 21 days. When compared to the control treatment, the findings of the processing phase showed that moisture percentages were identical for all treatments of soft cheese, but during the storage phase, a decline in moisture % was seen for all treatments. Regarding the total of acidity, it was observed that the treatments' values raised when the starter culture was applied. In all of the treatments, the pH values of the soft cheese that was generated were comparable. It is being researched right away after manufacture. In addition to enhancing the sensory , enhancing rheological, and physiochemical characteristics of the sample , the addition of starters enhanced the values of texture, such as hardness, cohesion, and elasticity. for soft cheese with a 45% total solids content

Keywords. Starters, Soft cheese, Dried sorted milk, Physiochemical properties.

1. Introduction

The cheese is highly valued, available in all regions and varied, as well as its flavour and consumer preference [1]. Although the temper industry has developed, in some countries it is still made by animal breeders in primitive ways and under underground conditions [2]. Cheese can be defined as the transformation of milk from a liquid state into a cohesive state called as a result of trimming the cover by means of a boom or something else, then separating the tracts and containing the resulting tracts on most of the fats and proteins and part of the orchids and cheating some salts [3] . Cheeses are classified into dry, semi-dry, dry, and very dry according to the moisture content [4] that offal is one of the most common dairy products in the world because of its multiplicity of types, as an important food, as well as its storage and transportation, as it reached world production in(1999). 1537000 tons from Iraq (28000) tons Cheese is one of the most important coagulated products at the appropriate temperature and humidity[5] , and it is also an ancient food that dates back to the foot of mankind, and

cheese is not only an essential source of protein, but it is the milky product that deserves more attention on it [6] It is one of the most important dairy products in most countries of the world, as the cheese industry dates back to more than 3000 years BC, after sheep were domesticated for the first time [7] . Many cheeses depend on production conditions and techniques that improve and adapt over time, as the main goal of cheese-making is to convert perishable milk into a product that can be stored for a longer period while preserving its nutrients [8].

2. Materials and Methods

2.1. Materials

Soft cheese was made from fresh full-fat cow's milk from Babylon Governorate's areas. Additionally, we used a microbial rennet made by the Japanese business LTD and a vegetable oil produced by Al-Ittihad Company in Babylon Governorate. Primer strains including:

- Streptococcus thermophiles and Lactobacillus acidophilus delbrueckii ssp. bulgaricus, a SACCO product, were also employed.
- Danisco produces Lactococ.cus lactis subsp. lactis and Lactococcus lactis subsp. Cremoris.
- A Bifidobacterium lactis isolate that Prof. Dr. Amer Abdul Rahman got from the College of Agriculture at the University of Baghdad.

2.1. Methods of Work

Making soft cheese by taking an amount of full-fat cow's milk, which was divided into two parts, one of which was left without adding starter culture and represented by the control coefficient, and the second part was added to it a mixture of starter cultures and dried full-fat milk, as well as vegetable fat, so it was used in the manufacture of soft cheese treats (T1 , T2, T3). All milk treatments were (pasteurized) at a temperature of 62.8°C for 30 minutes, after which the milk was cooled to 45°C than added starters at a ratio of (0.0105, 0.0335, 0.0027) g/L according to the regulations of the company producing each starter, then the mixture was incubated at a temperature of 42°C and left. for 60 minutes. Then microbial rennet was added in a specific proportion and mixed with milk. Then, it was immediately packed in the designated cans and left without stirring. Then, it was waited for coagulation about (30 minutes).

2.2. Physiochemical Tests for Soft Cheese

The pH of the cheese was estimated according to the above method. The percentage of moisture [9], ash [10] and fat [11] was estimated according to the mentioned method, while the percentage of total acidity was estimated according to the mentioned method [12] as for carbohydrates, their percentage was estimated mathematically according to what was stated in it [13] and protein was estimated according to the mentioned method [14] as follows:

$$\text{Carbohydrates} = (\text{Protein\%} + \text{Fat\%} + \text{Moisture\%} + \text{Ash\%}) - 100$$

2.3. Tissue Analysis

The texture parameters of the cheese were estimated using a texture analyzer (CT3, 4500 Brookfield engineering lab) with a load force of 5 kg, according to what was mentioned by [15].

2.4. Statistical Analysis

A complete random design (CRD) was used to study the characteristics of cheese during processing and ripening, and the significant differences between the averages were compared with the Least Significant Difference test (L.S.D). The program SAS (2018) was used in the statistical analysis of the data under scrutiny.

3. Results and Discussion

3.1. The Chemical Composition of Soft Cheese

The percentage of moisture for each soft cheese treatment is shown in Table 1; it reached 58.02% for the control treatment right after manufacturing, which is similar to what was discovered by [16], which was 59.00% for soft cheese, while it reached (58.70, 59.32, and 58.87)% for treatments (T1,T2,T3) respectively.

It should be observed that relative to the control treatment, the moisture was higher in the additional treatments, with treatment (T2) achieving the greatest moisture of 59.32%. The data also show that the humidity percentages for all treatments (C, T1, T2, T3) dropped during storage, with the percentages reaching 7 days after production being 57.88, 58.59, 59.51, and 58.71%, respectively.

Table 1. Chemical analysis of soft cheese for the control treatment and other treatments for soft cheese with different percentages of starter added immediately after manufacturing and during the storage period of 1, 7, 14 and 21 days at a temperature (4 $\pm$ 1 C) .

Treatment	Age of cheese	Moisture	Protein	Fat	carbohydrates	Ash	PH	Acidity ratio
C	1	58.02	16.09	16.39	7.19	2.31	6.72	0.16
	7	57.88	16.20	16.45	7.04	2.43	6.63	0.20
	14	57.71	16.27	16.51	7.02	2.49	6.17	0.31
	21	57.60	16.36	16.61	6.88	2.55	6.06	0.37
T1	1	58.70	14.16	23.42	1.34	2.38	5.91	0.55
	7	58.59	14.28	23.50	1.14	2.49	5.72	0.6
	14	58.42	14.51	23.59	0.83	2.65	5.53	0.69
	21	57.92	14.72	23.68	0.75	2.93	5.32	0.78
T2	1	59.32	12.97	22.92	1.97	2.82	5.7	0.59
	7	59.51	12.82	23.09	1.89	2.69	5.64	0.63
	14	59.65	12.93	23.24	1.65	2.53	5.2	0.69
	21	59.88	13.11	23.36	1.21	2.44	5.07	0.75
T3	1	58.87	14.25	21.43	3.08	2.37	5.66	0.40
	7	58.71	14.46	21.78	2.49	2.56	5.54	0.47
	14	58.59	14.60	21.97	2.11	2.73	5.41	0.54
	21	58.43	14.73	22.13	1.84	2.87	5.2	0.58

*Each number in the table represents an average of three replicates.

Findings of the statistical analysis show that there are no differences that are statistically significant at ($P \leq 0.05$) after manufacturing or over the 21-day storage period.

A rise in the protein % percentages of all treatments (C, T1, T2, T3) was seen during the storage period, with the percentages reaching 16.20, 14.28, 12.82, and 14.46%, respectively, seven days after manufacturing. However, after 21 days of manufacturing, the protein percentages reached (16.36, 14.72, 13.11, 14.73)% for all treatments. It has been observed that as the moisture content decreased and the protein % increased, the total solids, which includes protein, increased in the treatments to which the initiators were applied، and this result is near to what was found by [17] ,who indicated that the protein content is soft cheese ranges between (16.29_ 17.49) . A few percentages of whey proteins that are susceptible to heat treatment (pasteurization), which causes structural changes in them on the one hand, and their overlap with casein, where pasteurization results in denature, where they make up about 4-6% of the total protein content of soft cheeses, were also mentioned by [18].

As for the percentage of fat, the table shows the percentage of fat in the cheese of the different treatments previously mentioned, as the percentage of fat after manufacturing directly for the cheese of the control treatment C was (16.39)%, and this result is very near to what was found by[19] for the Iraqi soft cheese made from the milk of adult cows (16.67%), and differs from what was found by [20] for soft cheese of (18.0)%.

It is also noted from the results that there was a slight increase in the percentage of fat with the progression of the storage period for all treatments, as these values after 7 days for treatment C

reached (16.45)% and for other cheese treatments(23.50, 23.09, 21.78)%, respectively. The reason for this increase may be allocated to low humidity due to the evaporation of part of it during storage, which leads to an increase in total solids, including fat.
After 21 days of storage, however, the percentage of fat in the control treatment C was (16.61)%, and in the other cheese treatments to which a percentage of the starter culture was added, the percentage of fat was (23.68, 23.36, 22.13)%, respectively.
It is evident from the statistical analysis's findings that after 21 days of storage, there are no significant differences at the level ($p \leq 0.05$) in any of the transactions.
It is also noted from the results shown in the table above that there is an increase in the ash percentage of the treatment cheese with the addition of starters compared to the control treatment C, and the reason for this may be due to the fact that the addition of the starter leads to an increase in the solids. It is also noted from the same table that there is an increase in the percentage of ash during storage at a temperature of (4 ± 1) C. Where the percentage of ash in the cheese of the control treatment after 7 days of processing was (2.43)%, and this result is compatible with what was found by [21], who indicated that the percentage of ash in the soft cheese was (2.21)%, and for the cheese of other treatments, the percentage was (2.49, 2.69, and 2.56) %, respectively.
After 21 days of storage, the control C treatment's of cheese had an ash content of (2.55%), whereas the remaining treatments had ash contents of (2.93, 2.44, and 2.87)%. During storage, humidity causes a rise in the percentage of total solids, including ash which is one of its constituents.
And we can also notice a decrease in the percentage of carbohydrates after 7 days of processing, as the percentage of carbohydrates for the control treatment and for all treatments was(7.04, 1.01, 1.32, 2.49) %, respectively.

3.2. pH

The results shown in the table show the pH values of the different cheese treatments previously mentioned, where the PH values after manufacturing immediately for the control treatment C were (6.72), and this is consistent with what was found by [22]. 7 days for the control treatment C is 6.63 and for the other previously mentioned treatments (T1, T2, T3) it reached after 7 days of storage (5.91, 5.7 and 5.66), respectively. This is contrary to what was found by [23] , who indicated that the pH value of soft cheeses to which starter culture was added was (4.82).

3.3. Total Acidity Ratio

It is noted from the results that the total acidity values of all soft cheese treatments increased during the storage period. It reached (0.22)% after 7 days for the control treatment (C), and the eutrophic acidity value for the rest of the soft cheese treatments was (0.6, 0.63, 0.47), respectively. But after 14 days of the storage period, the pH values of the control treatment C reached (0.31)% and for the other treatments (0.69, 0.69, 0.54)%, respectively. Lactose converts to lactic acid as well as other organic acids, which decreases the Pka value inside the cell, and this makes the cell membrane more permeable to substances such as acetate and lactate, and this in turn leads to a decrease in the pH value and an increase in the total acidity of the product [24].

3.4. Rheological Tests

3.4.1. Hardness

Hardness was defined by [25] as the amount of force required to compress the sample and it is measured by applying a certain weight on the sample. Hardness was also defined as the force needed to achieve a certain deformation in the shape of the cheese sample [26]. The results shown in Figure (1) demonstrate the outcomes of the hardness test for the control treatment cheese C and coefficient cheese with starters added (T1, T2 and T3), respectively. Its value after manufacturing directly for the control treatment C was (598.9) gm, and for the control treatment and the other cheese treatments were (619.08, 618.9, 620.7) gm, respectively. The results showed an increase in the hardness values in all treatments to which the initiators were added after 14 days had passed during the storage period, where all the treatments reached (694.8, 649.81, 642.72, 656.98) gm, respectively, but after 21 days,

the hardness percentage of the control treatment reached (710.98) grams and other treatments)665.75, 660.78, 680.95) grams, respectively.

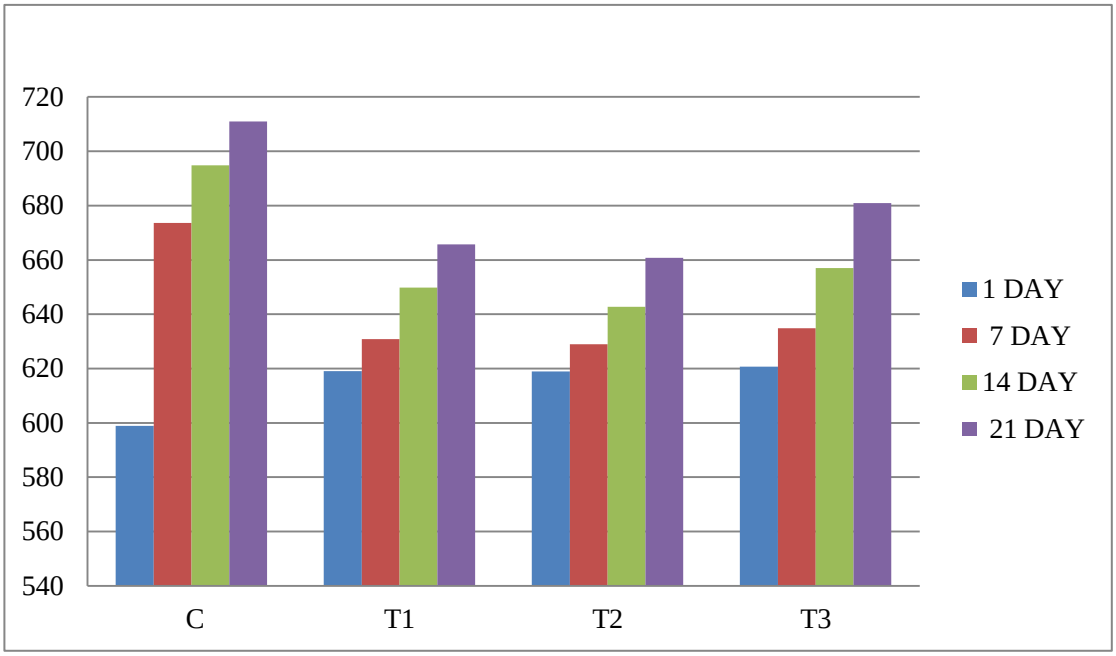

Figure 1. Hardness values for control treatment C and different cheese treatments immediately after manufacturing and during the storage period at (4 ± 1) C for 1, 7, 14 and 21 days.

It is clear that the addition of the starter culture led to an increase in the acidity of the cheese and a decrease in the pH, which affected the moisture content and then the hardness of the curd [27]. The reason for the increase in the hardness in other treatments may be attributed to the extent of the increase in the sub-correlations of casein with the increase in the ratio of casein to moisture.

3.4.2. Cohesion (Adhesion)

Cohesion is one of the most important properties of cheese tissue and depends on its acceptability from the consumer's point of view. Cohesion is defined as the forces of internal bonds that maintain the ideality of the product for the consumer, and it is expressed as the extent of deformation of the material when it is exposed to the cause of deformation before it is torn, and this depends on the nature of the protein [28]. The results shown in Figure(2) show the values of cohesion for cheese coefficients different. Its value after manufacturing immediately for the control treatment C was (0.49), and for the other soft cheese treatments, the cohesion values were (0.51, 0.53, 0.5), respectively.

After 14 days of processing, the value of cohesion for cheese treatment was (0.55) and for other treatments for soft cheese it was (0.58, 0.58, 0.54), respectively. From the results of the examination, an increase in the cohesion values is noted with the progression of the storage period, as the cohesion value reached after 21 days for the cheese treatment. C is (0.57) and the other coefficients for soft cheese were (0.6, 0.59, 0.57), respectively.

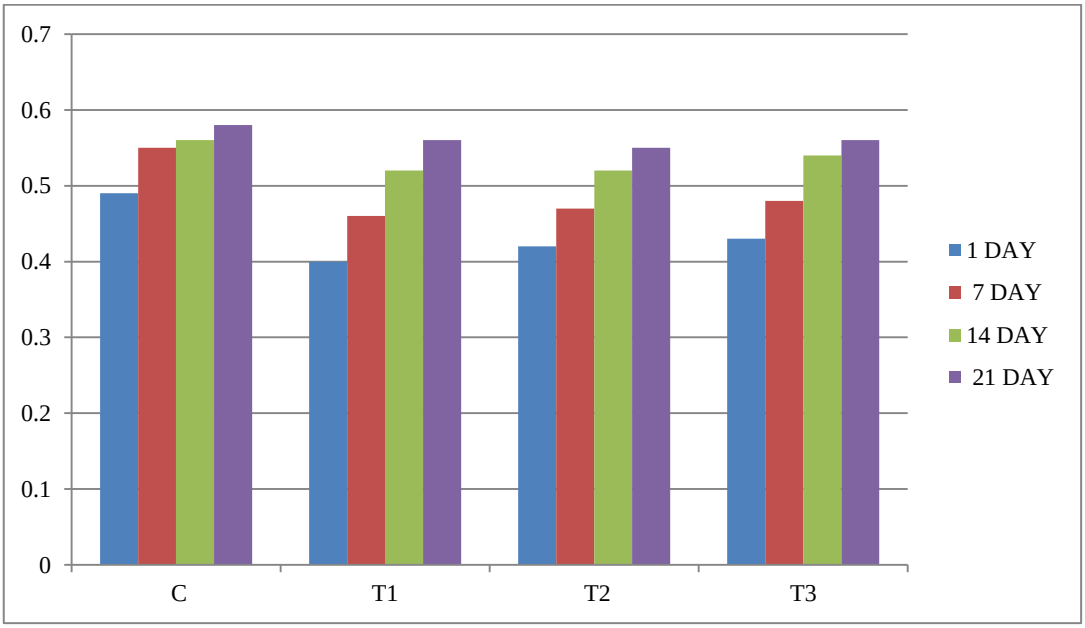

Figure 2. Cohesion values for control treatment C and different cheese treatments immediately after manufacturing and during the storage period at (4 ± 1) C for 1, 7, 14 and 21 days.

The results showed that the coefficients to which the initiators were added were of higher cohesion, and this is consistent with what he mentioned [29]. It is noted from the statistically analyzed results that there are no significant differences at the level ($P \leq 0.05$) between the cohesion values of all treatments with the addition of starters compared to the control treatment after storage for 21 days of manufacture.

3.4.3. Flexibility

The results shown in Figure (3) illustrate the values of elasticity for each of the cheese of the control treatment and the cheese of the treatments to which the starters (T1, T2, T3,) were added, as its value after manufacturing directly for treatment (C) was (9.5) mm and the other soft cheese treatments reached values (9.4, 9.1, and 9.6) mm, respectively.

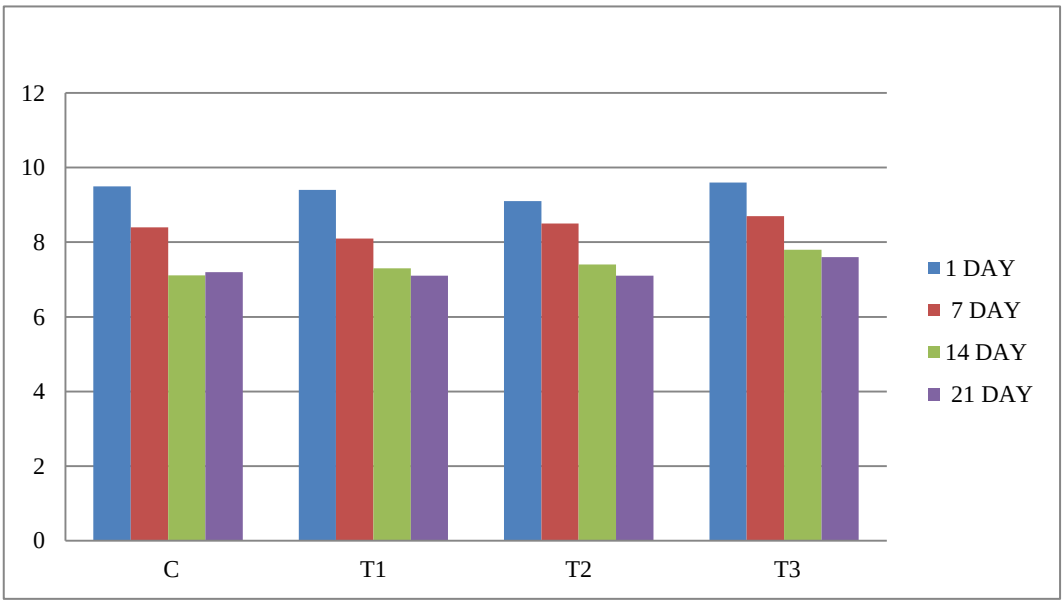

Figure 3. Elasticity values for control treatment C and different cheese treatments immediately after manufacturing and during the storage period at (4 ± 1) C for 1, 7, 14 and 21 days.

It is noted from the statistically analyzed results that there are no significant differences ($p \leq 0.05$) compared with the control treatment after manufacturing between all other treatments directly. It is also noted from the results a decrease in the elasticity values in other treatments for soft cheese, and this reason is due to the increase in solids that leads to a lack of moisture and then a decrease in elasticity in these treatments after 14 days of manufacturing, so the value of elasticity for treatment C (7.11) For other soft cheese treatments, the value of elasticity was (7.3, 7.4, 7.8) mm. This decrease may be due to a decrease in the moisture content during storage due to the exudation of a small part of the whey, as well as the evaporation of a part of the moisture, which leads to an increase in the percentage of solids. Hence the lack of flexibility and it is noted from the statistically analyzed results that there are no significant differences ($p \leq 0.05$) for the treatments with added starters compared with the control treatment C, but it is noted that there are significant differences for all treatments at the end of the storage period of 21 days compared with the same results for the single treatment after manufacturing directly.

3.5. Sensory Evaluation

The results in Table (2) clarify the outcomes of the sensory evaluation of the characteristics of taste, flavour, texture, bitterness and color of soft cheese manufactured from different treatments at the age of 1, 7, 14 and 21 days from manufacturing. Additionally, the findings demonstrated that there are no appreciable variations between any of the treatments in the mean ratings given for the color characteristic just after manufacture. Additionally, it should be highlighted that there is a decline in the sensory evaluation scores for the color characteristic with storage, as it was given to treatment (C) (9) degrees after 7 days rather than (8.1, 9, 9), as it was on the first day of manufacturing.

After 21 days of production, the control treatment (C) had a color characteristic of seven degrees, whereas the other treatments for soft cheese had color characteristics of seven, eight, and thirteen degrees, respectively.

Table 2. Sensory evaluation of soft cheese for the control treatment and other treatments of soft cheese to which different proportions of starters are added immediately after manufacturing and during the storage period of 1, 7, 14 and 21 days at a temperature of (4 + 1 °C).

Treatment	Age of cheese	Color 10	Flavor 10	Cohesion 10	Textures 10	Gallbladder 10	Openings 10	Total Scores Out of 60
C	1	10	9	8	8	10	10	55.0
	7	9	8.5	8	8.14	9	9.3	51.94
	14	8	7.3	7.6	8.10	9.35	9.1	49.45
	21	7	6	7.1	8.2	9.32	9.4	47.02
T1	1	9	8	8.5	8.27	10	10	53.77
	7	8	7.7	7.9	7.8	9	9.8	50.2
	14	7	7.4	7.7	7.5	9	9.6	48.2
	21	7.2	7	7	7	8	9	45.2
T2	1	10	8.5	9	9.3	10	10	56.8
	7	9	7.8	8.1	9.1	9.5	9.8	53.3
	14	8.3	7	7.6	8.8	9.1	9.5	50.3
	21	8	6.9	7.1	8.4	8.8	9.3	48.5
T3	1	10	9	10	9.6	10	10	58.6
	7	9	8.7	9.9	9.06	9	9.5	55.16
	14	8.25	8.3	9.5	9.15	8.3	9.1	52.6
	21	8.13	7.9	8.9	9.1	8.11	8	50.14

*Each number represents an average of three replicates.

It can be noticed from the above table that the scores awarded to the flavor characteristic were higher for the treatments to which the starter was added, as the scores granted after 7 days for treatment (C) were (8.5) degrees. As for the other treatments for soft cheese, they were (7.7, 7.8, 8.7) degrees,

respectively According to what he referred to [30]. The flavor of the cheese is a synthesis of stimulation for the senses of taste, smell, and chemical compositions. The raw materials and production and processing techniques utilized, such as pasteurization and naturalization, as well as the chemical and biological changes that take place during ripening, are all impacted by this flavor. Regarding the degrees awarded for the characteristic of cohesiveness, the control treatment (C) received (8) degrees, while the other treatments of soft cheese received (7.9, 8.1, and 9.9) degrees after 7 days of storage.

After 21 days, the other soft cheese treatments received scores for the characteristic of cohesion that were (7, 7.1, and 8.9) degrees, respectively , and the control treatment (C) received a score of (7.1). However, it can be seen from the statistical analysis of the results that there were no significant differences between any of the other cheese treatments. When it comes to the soft, the other cheese treatments received grades for the texture characteristic after 7 days of storage that were (7.8, 9.1, and 9.06) degrees, respectively, while the control treatment (C) received a grade of (8.14) degrees. We can also see the results for the texture characteristic after 21 days from the table. The score was provided to the control treatment (C), which was (8.2) on one day of the storage period, and the texture characteristic (7, 8.4, and 9.1) degrees, respectively, was given the score for the other cheese treatments.

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The Serum Testosterone, Zinc Levels and Testicular Tissue Developments in Male Lambs: the Effect of Using Nano Selenium and Powdered Cannabis Seeds as a Dietary Supplement

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Abstract. Cannabis sativa L. is regarded as a dietary plant. Typically, whole cannabis seed has about 25% protein and about 30% oil (3% saturated and 28% unsaturated fatty acids). Nano selenium is an important element that can perform bioactivity, increase digestibility, and have a crucial role in reproduction in livestock. Due to the physiological importance of cannabis seeds and nano selenium, this study conducted the effect of both on testosterone hormone, serum zinc, and testicular histology. The study included 20 male Karadi lambs with an average age of 3 to 4 months. Lambs were divided into four groups. In the second group, 0.5 mg/kg fed of nano selenium was administered orally, while the first group served as the control group. A mixture of 0.5 mg and 250mg/kg fed of nano selenium and powdered whole cannabis seeds, respectively, were administered orally to the fourth group. In comparison, 250 mg/kg fed of powdered whole cannabis seeds were supplied to the third group. Gelatinous capsules were prepared for nano selenium and powdered cannabis seeds to ensure daily intake of the exact amount of nano selenium and cannabis seeds powder. The prepared gelatinous capsules were administered orally daily at 9:00 am up to 60 days. Serum zinc levels and Testosterone were increased significantly ($p<0.05$) in administrated nano selenium group compared to the control group. In the third and fourth groups, Significant changes were seen in the serum levels of zinc and testosterone ($p<0.01$). Testicular histology in the nano selenium group was scored ten according to Johnsen criteria, as seminiferous tubules were normal. Complete spermatogenesis with fully developed sperm cells has been observed, as well as the existence of spermatocytes and healthy sperm cells or spermatids. In third and fourth groups scored five according to Johnsen criteria, as no sperm cells or spermatids appeared, there was the presence of spermatocytes, necrotic cells of Sertoli cells, and no spermatids. This study aimed to potentially apply cannabis seed powder and nano selenium as a food additive in 0.5 mg/kg fed and 250 mg/kg fed, separately or both together, to increase the male lamb's sexual development and maturity.

Keywords. Cannabis seeds, Nano selenium, Testosterone hormone, Serum zinc, Seminiferous tubules, Testicular histology.

1. Introductions

Cannabis seeds have been used since ancient times as a significant supply of plant fiber for industrial purposes. Despite this, there has been increased interest in hemp seed over the past few decades. Although it is officially an achene, the hemp seed is a one-seeded dry fruit that resembles the cereal caryopsis in that the pericarp is loosely attached to the seed [1]. Hemp seeds produced for fiber production were typically viewed as waste materials and, at most, mainly used as animal feed [2]. The hemp seeds that are planted to make fiber are typically regarded as waste materials, and at most, they have been fed to animals. However, in recent years, as awareness of the health advantages and beneficial uses of hempseeds has grown, These seeds are now a more popular product with a significant and growing market due to increasing manufacturing. [3, 4]. Due to its high nutritional characteristics, hempseed has historically been regarded as one of the most nutrient-dense foods. In addition to its processed goods like canvas, wheat, and protein grease paint, it sells whole or unshelled seeds (hempseed kernel). Even though numerous studies have noted excessive variability in hempseed composition, it typically contains 20–25 lipids with a distinct fatty acid (FA) composition, 20–25 proteins that are smooth to condensation and fat in essential amino acids, and 20–30 carbohydrates, of which a sizable portion may be made up of nutritive fiber, especially unavoidable, as well as vitamins and minerals [5, 6]. Hempseed oil has high levels of polyunsaturated fatty acids (PUFAs) and saturated fatty acids (SFAs). More specifically, hempseed oil contains 70 to over 80 polyunsaturated fatty acids (PUFAs) out of up to 90 unsaturated fatty acids, mainly depending on Genotype and environmental conditions [7, 8]. According to their nutritional requirements, minerals can be divided into macro-factors (i.e., minerals wanted in a quantity of >50 mg/day), such as phosphorous, potassium, magnesium, calcium, and sodium, or micro-factors or hint factors (i.e., minerals wanted in a quantity of 50 mg/day), such as iron, manganese, copper, and zinc [5, 9-11]. Selenium has been shown to increase the fertility of dairy sheep. Animal fertility increased when selenium supplements were added to pastures with extremely low selenium levels [12]. Selenium has a connection to both animal and human reproduction. Selenium is crucial for sperm synthesis, testosterone generation, placental retention, and fertility. Selenium deficiency has been associated with stunted growth and diminished fertility. Changes in the Leydig cells' luteinizing hormone receptors, which have an impact on testosterone production, were used to identify selenium deficiency. In prostate cancer patients, selenium decreases the growth of cancer cells by inhibiting the creation of RNA, DNA, and proteins. It has been demonstrated that selenium affects the testis' general morphology [13]. Being significant in cattle and sheep (lamb) is conceivable with Neonate calf frailty, calf scours, pneumonia, stinginess, low weight growth, retained placentas, stillbirths, abortions, impaired fertility, reduced fertility, numerous births, and decreased wool production are all symptoms of selenium deficiency. Numerous swine diseases, such as nutritional myopathy, mulberry heart disease, dietary hepatitis, stomach ulcers, diarrhea, and piglet weakness at birth, have been connected to selenium deficiency [14, 15]. The purpose of the present investigation is to evaluate the effect of dietary nano selenium and cannabis seeds powder on reproductive developments, sexual hormone production, and testicular histology in male lambs.

2. Material and Methods

Four sets of twenty male Karadi lambs, all around 3 to 4 months old, were created. Each lamb received its cage and separate feeding. The feed was prepared following the accepted protocol. Wheat, soybean meal, yellow maize, rye, minerals, and salt made up the target diet, whereas wheat straw made up the baseline diet. Food was given out once daily at 9:00 am in portions assessed to represent 3% of live body weight (LBW) to support maintenance and daily benefit. The first group sat as the control group, the second received a capsule containing nano selenium, the third received a capsule containing powdered cannabis seed, and the fourth received a capsule containing both nano selenium and powdered cannabis seed. Animals received 0.5 mg/kg/day fed of nano selenium, 250 mg/kg/day fed of cannabis seeds powder, and 0.5 mg/kg/day fed mixed with 250 mg/kg/day fed of nano selenium and cannabis seeds powder up to 60 days. Lambs were fed for ten days prior to the experiment's start without receiving any treatment to help them adjust to the new surroundings. Each lamb was given a subcutaneous injection of 1 ml of BULITEL (Ivermectin with Closantel Sodium 1%+10%,

Rongchang, China: Chongqing BULL Animal Pharmaceutical) to treat both internal and exterior parasites. Entrotoxia-blackleg was treated with the purified vaccine SYMTEROVAC (Biopharma, Morocco). 1 cc of the second dose was given subcutaneously after 30 days. The Wuhan Dongxin Mill Imp and Exp Trade Co, Ltd. is a company that manufactures nano selenium particles, which are imported from China. It is kept in an enclosed, dry environment and is 99.99% pure black powder. The Bagdad University/College of Education for Pure Science/Ibn Alhaitham central lab validated a sample of nano selenium particles for close to 2-3 gm from the material utilizing an X-Ray Diffraction.

It has been demonstrated that hexagonal selenium nanoparticles, in particular, and the crystal lattice constants of a=b=4.3662 and c: 4.9536, are present in nano selenium patterns and are polycrystalline. Debye-equation Scherer's was used to compute the average crystal size for the prevailing trend, and the result (101) indicates that the average crystal size is almost (15nm) [16]. The PK Cannabel firm in Moscow, Russia, provided the cannabis seeds. To create a powder, seeds were crushed using a specialized grinder. Whole cannabis seeds were used in this study. Cobas e411 (Roche, Germany) was used for the estimating of serum testosterone hormone. The reagent contained Preservative microparticles coated with streptavidin, 1 bottle, 6.5 mL; transparent cover; Microparticles with streptavidin coating, 0.72 mg/mL (M). Biotinylated monoclonal anti-testosterone antibody (sheep), 40 ng/mL; 2-bromoestradiol; MES buffer, 50 mmol/L, pH 6.0; preservative; Anti-testosterone-Ab-Biotin (gray cap), 1 bottle, 10 mL (R1). Testosterone-peptide-Ru(bpy)2+, 1 bottle, 9 mL: Preservative, MES buffer 50 mmol/L, pH 6.0, and a testosterone derivative labeled with ruthenium complex at 1.5 ng/mL. (R2). Serum zinc level was determined using a colorimetric method, the LTA s.r.i Zinc kit (Milan, Italy) and the optima SP-300 spectrophotometer (Tokyo, Japan) were used to measuring the level of zinc in the serum. The reagent's ingredients were Dimetilgioxime 1.25 mM, Saliciladoxime 12.5 mM, Borate buffer 0.37 M, pH 8.2., surfactants, and preservatives. Chemical agent A NITRO-PAPS; preservatives, 0.4 mM. Resistant B 200 g/dl (30.6 mol/l) of zinc ion; stabilizers and preservatives. (Standard). Gathered and divided into 1 cm^2 pieces. The samples had 10% formalin added to them. Utilizing a LEICA TP 1020 automated Tissue Processor, the tissues were processed (Heidelberger, Nussloch, Germany 2018). The fixation, dehydration, and infiltration of histological tissue samples can all be automated using the Leica TP1020 Tissue Processor.

In order to the section: 5 µm thick pieces of paraffin block were cut using a rotary microtome (Accu-Cut SRMTM 200, Sakura Finetek Europe B.V.) and mounted on a microscope slide. Hematoxylin and eosin were used to stain the tissues (H&E). Using the IBM SPSS statistic 26 application, the results were statistically examined. ANOVA and T-test were the statistical analysis methods employed.

3. Results

The results showed that nano selenium and cannabis seeds impacted testosterone and serum zinc levels in male lambs. Table 1 and figure 1; in contrast to the control group, serum zinc levels rose in all treated groups. The impact of orally administrated nano selenium on the lambs on serum zinc level was altered ($p<0.05$). The serum zinc level was also elevated in lambs administrated with cannabis seeds powder and also cannabis seeds powder in combine with nano selenium ($p<0.01$). Testosterone level in the serum was also altered in all treated groups in comarison to the control group, as shown in table 1 and figure 2. In nano selenium was elevated ($p<0.05$), and in cannabis seeds, powder and cannabis seeds were combined with nano selenium ($p<0.01$).

Table 1. Serum zinc levels and Testosterone for male lambs orally administrated with Nano selenium, Cannabis seeds powder, and Nano selenium mixed with Cannabis seeds powder compared to the control group. The table represents mean ± standard deviation.

Groups	Zinc (µg/dl)	Testosterone (ng/ml)
Control	219.33±0.57	5.69±0.09
Nano selenium	259.0±15.71^{*a}	6.03±0.18^{*b}
Cannabis seeds	289.33±6.5**	7.25±0.08^{**c}
Nano Selenium and Cannabis seeds	299.33±9.50^{**b}	7.95±0.04**

* P<0.05, ** P<0.01. Indicates the level of significant differences compared to the control group. a, b, c indicates significant differences between groups in the same column.

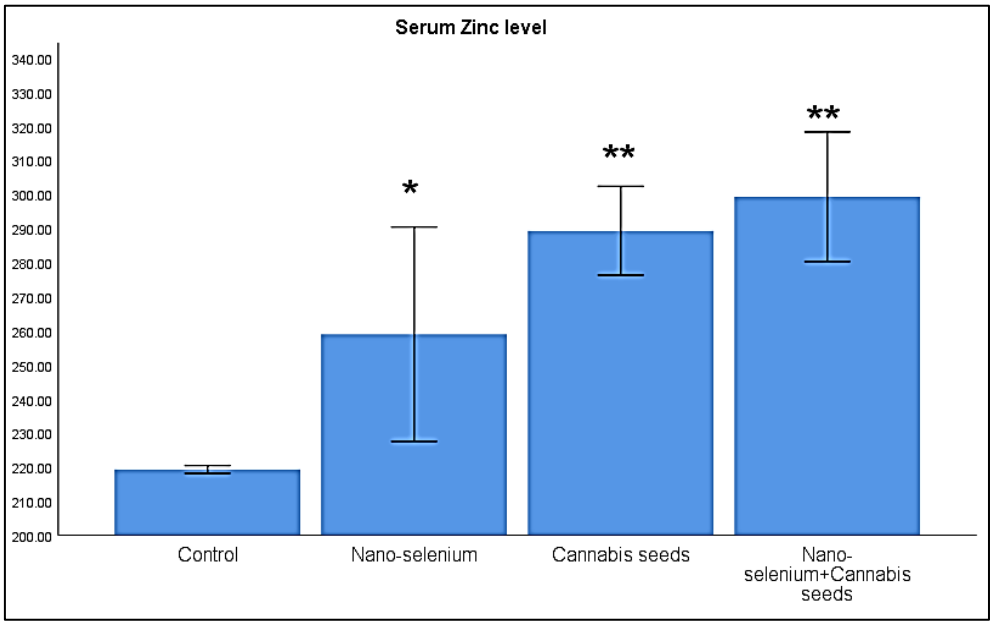

Figure 1. Serum zinc levels (µg/dl) orally administrated with nano selenium, Cannabis seeds powder, and nano selenium with Cannabis seeds powder compared to the control group. The table represents mean ± standard deviation. * P<0.05, ** P<0.01. Indicates the level of significant differences compared to the control group.

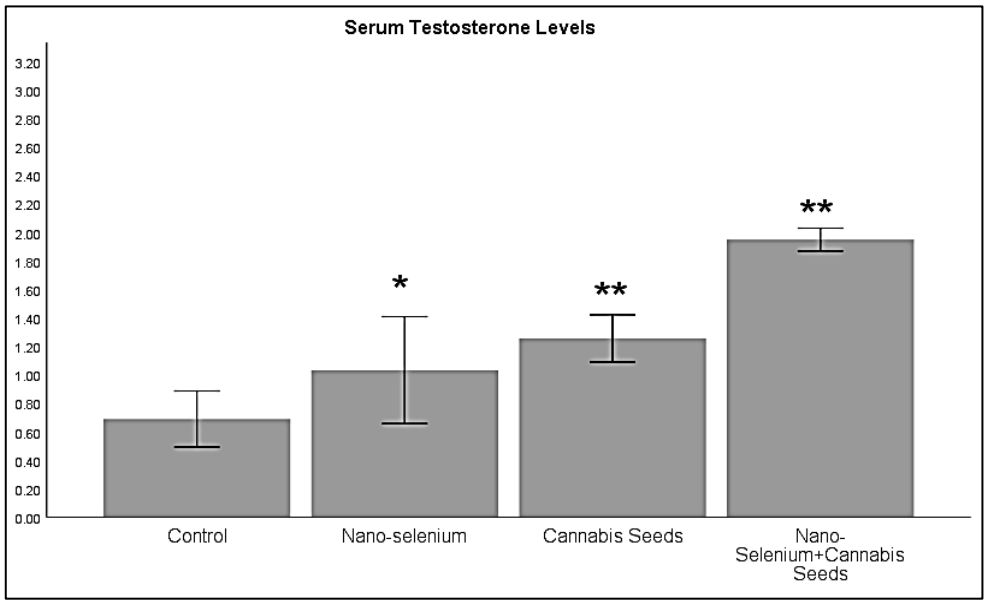

Figure 2. Serum testosterone level (ng/ml) for male lambs orally administrated with nano selenium, Cannabis sees powder, and nano selenium with Cannabis seeds powder compared to the control group. The table represents mean ± standard deviation. * P<0.05, ** P<0.01. Indicates the level of significant differences compared to the control group.

The histology examination was obtained in order to investigate the impact of cannabis seeds powder and nano selenium on testicular tissue. There are several classification schemes described based on the five primary histological patterns of spermatogenesis, including Tubular sclerosis, which is the absence of seminiferous tubules; the Sertoli cell-only syndrome, which is the absence of germ cells within the seminiferous tubules; the spermatogenic arrest, which is the lack of progress past the spermatocyte stage; and, finally, the presence of all germ cells in a single biopsy, these several stages of spermatogenesis frequently coexist in practice (mixed pattern). Because of this, spermatogenesis is

classified quite differently by different pathologists, which reduces the diagnostic and prognostic utility of testicular biopsies [17]. In this study, the testicular tissues at five µm thick and 400X magnified used hematoxylin and eosin for staining the tissues, and Johnsen criteria for evaluating the effect of nano selenium and cannabis seeds powder on male lambs' testicular tissues were scored. The Johnsen criteria convert the cell profile found along the seminiferous tubules into a ten-point score system for assessing spermatogenesis. Johnsen scores range from 1 to 10, with 1 representing a complete absence of germ cells and 10 representing maximum spermatogenesis activity [18].

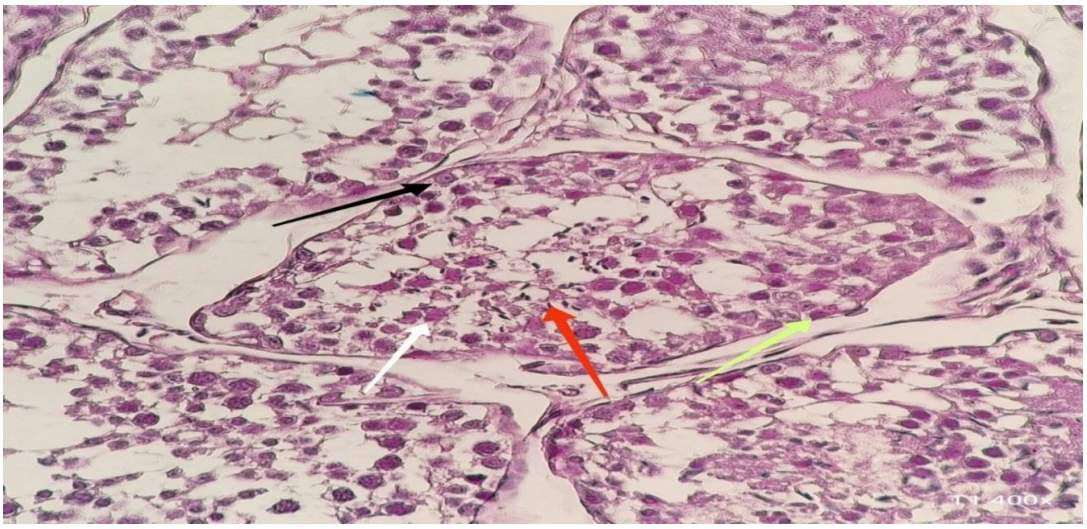

Figure 3. Seminiferous tubules in a testis segment from the control group are seen in a normal histological manner. Scores equivalent to Johnsen: 10 (Complete spermatogenesis with mature sperm cells). White arrow (spermatocytes), a red arrow (Sertoli cell), a green arrow (spermatogonia), and a black arrow (Sertoli cell) (spermatids).

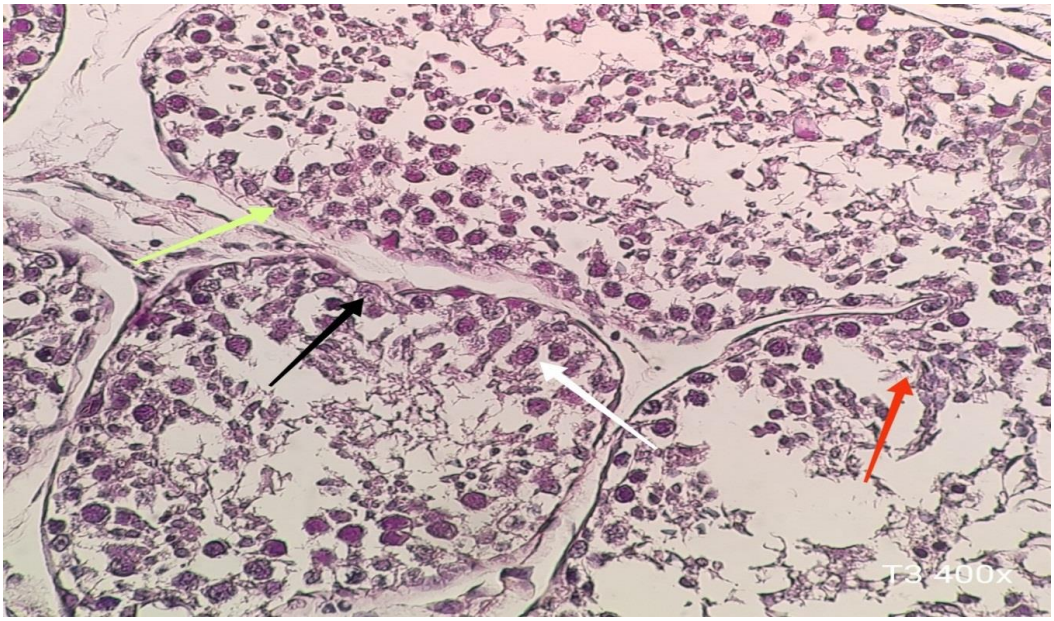

Figure 4. Testicular tissues from male lambs were orally administrated with 0.5mg/ kg /day of fed nano selenium. Seminiferous tubules in a testis segment in the nano selenium group normally seem histologically in the photomicrograph. Scores equivalent to Johnsen: 10 (Complete spermatogenesis with mature sperm cells). White arrow, a green arrow (spermatogonia), and a black arrow (Sertoli cell).

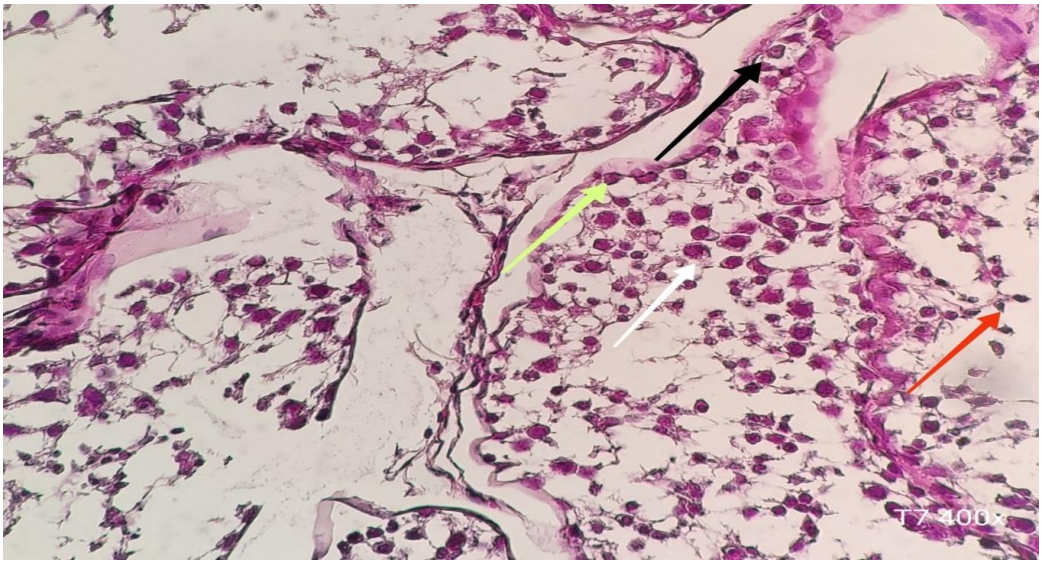

Figure 5. Testicular tissue from male lambs orally administrated 250 mg/kg/ day of fed cannabis seeds. A photomicrograph of a bunch of cannabis seeds reveals the disappearance of seminiferous tubules' typical histological appearance. Scores akin to Johnsen: 5 (No spermatids or sperm cells, presence of spermatocytes). White arrow (spermatocytes), a red arrow (Sertoli cell), a green arrow (spermatogonia), and a black arrow (Sertoli cell) (necrotic cells, no spermatids).

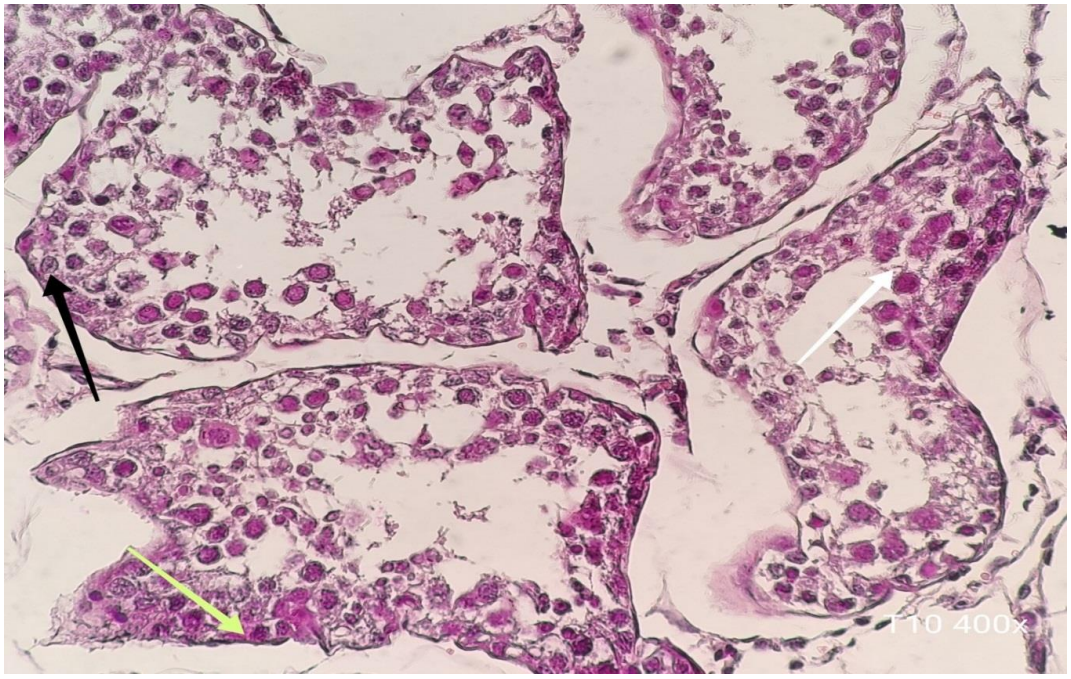

Figure 6. Testicular tissue from male lambs administrated orally 0.5 mg/kg of fed nano selenium + 250 mg/kg/day of fed cannabis seeds powder. Scores akin to Johnsen: 5 (No spermatids or sperm cells, presence of spermatocytes). Sertoli cells with a black arrow, spermatogonia with a green arrow, spermatocytes with a white arrow, and no spermatid.

4. Discussion

The synthesis of proteins, the development of new cells, the production of DNA, the repair of damaged tissue, and the maintenance of a healthy immune system all depend on zinc. Enough zinc is required during times of rapid growth because it promotes cell growth and multiplication [19-22]. Due to the importance of zinc in developing male reproductive and maturity, it was important to estimate the serum zinc level after using nano selenium and cannabis seeds orally. Nano selenium affected the

level of zinc in serum in this study which agreed with the other studies that animals treated with nano selenium and selenium particles have higher levels of zinc and other minerals [23]. This may be related to the impact of nano selenium on dietary mineral absorption, particularly zinc. The zinc level was dramatically changed in the cannabis seeds group and the nano selenium + cannabis seeds group. There is no single study on the influence of cannabis seeds powder and a mixture of nano selenium together with cannabis seeds on the level of zinc. The alteration of zinc level in the serum may be due to the cannabis seeds components which contain major macro-elements, including zinc. A different study was conducted on the amount of zinc in the cannabis seeds component, estimated to be around 10-11 mg/100 g of cannabis seeds [24].

Economically the fertility rate in livestock is very important so dietary supplements can be used [25]. Studies on the male hormone (Testosterone) and testicular tissue development are necessary to evaluate dietary supplements' effect on male reproduction. Nano selenium as a supplement has positive feedback on male reproductive development. Many studies have shown the impact of selenium nanoparticles on male reproduction in livestock, which appears to be necessary for healthy spermatozoa formation in both experimental and livestock animals, as well as likely in humans [26, 27]. This study agrees with the other studies that nano selenium has an improving impact on the development of testicular maturity and function and increases the level of testosterone hormone [28]. Many studies found dealing with the impact of dietary cannabis seeds on testicular functions and the level of testosterone hormone in experimental and farm animals such as poultry, rabbits, and ruminants, as well as rats [29-31]. In our study, the number and activity of testis somatic and germ cells may increase if these hormone levels rise significantly, which would increase the weight of the epididymis and testis because testosterone promotes the growth and secretor activity of the reproductive organs [32, 33]. via stimulating the Leydig cells' production of testosterone, luteinizing hormone (LH) indirectly promotes spermatogenesis by influencing the peritubular cells and the Sertoli of the seminiferous tubules. Other research indicated that the cannabis seed extract had a favorable effect on LH hormone levels, which might boost the amount of testosterone Leydig cells secrete [32]. The increasing effect of cannabis seeds on the level of testosterone hormone may link to the presence of omega 3 and omega 6 as they are rich in cannabis seeds; since many studies have examined the significance of the ratio of omega-3 to omega-6 on spermatogenesis, the histological results in this study may be related to the high omega-3:6 ratio of polyunsaturated fatty acids in cannabis seeds [34, 35]. Numerous pieces of research have examined the beneficial link between PUFAs (polyunsaturated fatty acids) and the capacity to reproduce. Feeding PUFAs to animals changed the PUFA makeup of their testicles. In order to better understand the beneficial role that PUFAs play in animal reproduction, various studies have been conducted [36].

Most of these studies have focused on the effects of fish oil or other diets high in long PUFAs on adult male animals. According to the findings of this study, early testicular development and increased testosterone levels are necessary to boost lamb reproductive success. However, cannabis seeds are rich in omega 3 and omega 6, and PUFAs. However, the result of this study shows the seminiferous tubules in the cannabis seeds were negatively impacted, and they lost their typical histological appearance. Due to the spermatocytes' presence despite the lack of sperm cells or spermatids, we received a Johnsen-like score of 5. Visible Sertoli cells, spermatogonia, and spermatocytes may be seen. Necrotic cells prevent the production of spermatids and sperm. The effect of cannabis seeds on testicular histology in other studies agreed with the result of this study. There is limited study on the impact of cannabis on testicular histology in lambs, but the investigation on male rats showed results that agreed with this study [37]. The motility of sperm is one of the sperm functions that cannabinoids adversely affect. Cannabinoids function on sperm via activating CB1 receptors, which are found in mature sperm, to reduce these inhibitory effects. Impacts of cannabis on sperm motility, it appears that, at least in laboratory settings, activation of CB1 receptors reduces sperm motility in both vertebrates and invertebrates. CB1 receptors are expressed in germ cells from the spermatogonia to the mature sperm by the research that is now available regarding animal studies. According to investigations, this receptor can be found in the spermatogonia, primary spermatocytes, and sperm of rat testis [38]. Using oral administration of 250 mg/kg/day of fed cannabis seeds for 60 days in lambs

may increase the number of cannabinoids in the body and eventually have a negative impact on testicular tissue and development.

Conclusion

Orally administrated 0.5 mg/kg/day fed of nano selenium and 250 mg/kg/day fed of cannabis seeds for 60 days have a good impact on increasing serum zinc levels. Nano selenium increases zinc absorption, and cannabis seeds have a good amount of zinc in its component. Nano selenium has a role in increasing testosterone levels and testicular development as well as cannabis seeds because of the rich component of PUFAs and the amount of omega 3 and 6 and their ratio. Using 250 mg/kg fed daily administrating to male lamb's cannabis seeds has a negative impact on testicular histology due to the accumulation of cannabinoids, which is a component in cannabis seeds.

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Effect of some Medicinal Plant Extracts and Chemicals on Blood Parameters of Female Rats

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Abstract. The study was carried out on 45 sexually mature female albino rats At the age of 7-8 weeks and weighing 160-180 ± 10 gm., nine treatments were used in this study, Where five rats were isolated for control treatment (without infection),The remaining rats were injected (Subcutaneous) with Indian-made Alloxan, Which was prepared at the time of injection at a dose of 100 mg/kg of body weight. The purpose of this study was to determine the effect of extracts of medicinal plants (Curcumin, Origanum majorana, and Vitex aguns-castus) at 50-100 mg/kg body weight and chemotherapy (metformin at 50 mg/kg body weight) on blood images and biochemical characteristics. The results showed a significant decrease in WBC and FBS in all treatments compared to T2 (diabetics without treatment). T2 had a significant decrease in the globulin characteristic when compared to the other treatments. As for hemoglobin, it was noted that T5 was superior to T3. It was noted that T8 had a significant superiority over T2 and T3 in the MCHC trait, while no significant difference appeared between the treatments in the RBC, PCV, MCH, total protein, and ALB traits. It is clear from the results of the current study that the aqueous extracts have a positive effect on blood images and biochemical characteristics in female rats.

Keywords. Curcumin, Origanum majorana, Vitex aguns-castus, Metformin and biochemical characteristics.

1. Introduction

Diabetes mellitus is a collective term for heterogeneous metabolic disorders [1,2], and one of the most prevalent diseases worldwide. A global study [3], found that less than half a billion people live with diabetes worldwide. The number is expected to increase due to the global prevalence of diabetes in 2019 by about 9.3% (463 million people). It will rise to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045.There is an Iraqi study by a group of scholars [4], in which they mentioned that about 1.4 million Iraqis suffer from diabetes; the prevalence rate of the disease according to age ranges from 8.5% to 13.9%; and a local study that included more than 5400 people in the city of Basra, southern Iraq, reported a prevalence of diabetes of 19.7% in people between the ages of 19 and 80. It is expected that it will infect 693 million adults by the year 2045 [5], so the use of medicinal herbs has spread widely at the present time as it treats many diseases and is almost free of harmful side effects [6]. Curcumin is found in different types of turmeric and is considered the best natural source. Curcumin is found in turmeric at a rate of 3.14% of its total weight. It is also found in Amada turmeric or the so-called "mango ginger," which is from the ginger family and has a flavor similar to that of

mango, which is why it was given this name [7]. Curcumin contains many effective compounds, the most important of which are phenols, which are considered excellent antioxidants [8].
Previous studies have shown that dietary curcumin inhibits blood glucose levels in diabetic patients and their animal models [9] and [10]. An experimental study also showed the effectiveness of turmeric on reducing blood glucose levels in white rats, and this was through its activation of pancreatic cells in stimulating insulin production [11]. In his study, he found that curcumin reduces complications of diabetes. Origanum majorana is a perennial herbaceous plant, 30-60 cm high. Its stem is rigid, ribbed, and covered with fine hairs. Its color is brown at the top, mixed with red. The leaf is tongue-shaped. Its flowers are white, tending to pink. It is native to Turkey and Cyprus, and it has spread from there to countries in the Mediterranean basin, such as Lebanon, Hauran, southern Syria, Iran, North America, the Arabian Peninsula, and India [12]. Origanum majorana contains phenolic acid, which is the main chemical constituent in origanum majorana [13]. It also contains flavonoids, tannins, sterols, and triterpenes [14].The effect of this plant is attributed to its ability to restore the function of pancreatic tissue by causing an increase in insulin production, inhibiting intestinal absorption of glucose, or facilitating the metabolism of insulin-dependent processes [15]. In their study, [16] indicated the possibility of treating diabetes by reducing blood sugar by using marjoram leaf extract because it contains flavonoids and phenolic compounds, in addition to improving metabolism and reducing oxidative damage.Vitex aguns-castus herb, also known as vitex aguns-castus herb, has its origins in the Middle East and Central Asia. The digestive system, as well as the treatment of mood swings and depression symptoms associated with the menstrual cycle, aids in the treatment of infertility in women caused by hormonal and ovulation disorders [17]. The palm of the vitex aguns-castus contains one flavonoid compound, isovitexin, and four flavonolic compounds: campferol, ramnoglucoside, quercetin, and rutin. an amino acid, including alanine, arginine, proline, phenylalanine, methionine, and choline, in addition to alkaloids, coumarins, and silsicolite [18].

2. Materials and Methods

The study was conducted in the animal house in the College of Veterinary Medicine, University of Tikrit, for the period from 19/4/2022 to 18/5/2022 and was done through the following:

2.1. Sample Collection and Preparation

The drug metformin, which originated in a French province, was purchased from a pharmacy in Salah al-Din-Tikrit. Extracts (curcumin, origanum majorana, and vitex aguns-castus) were also purchased locally, with the Al-Emad Company in Iraq, and from abroad, with the Al-Emad Company in the United States.

2.2. Animals used in the Study

The white laboratory rat, Albino, was used at the age of 7-8 weeks with a weight of 160–180 g ± 10 g. In this study, 45 sexually mature females were used, obtained from the animal house of the College of Veterinary Medicine at the University of Tikrit, and they were monitored for two weeks before the start of the experiment to acclimatize and ensure good health, her health condition, and the fact that she is not pregnant. They were also examined by a veterinarian specialized in the center to ensure that she is safe, healthy, and free from diseases before using her in the experiment.

2.3. Experiment Design

2.3.1. Induce Diabetes Mellitus

Five rats were isolated for control treatment (without infection), and the remaining rats were injected subcutaneously with Alloxan of Indian origin, which was prepared at the time of injection at a dose of 100 mg/kg of body weight. as it was dissolved by 1 gram of alloxan in 10 ml of physiological solution (normal saline) after starving the animals for 12 hours [19] and [20], and after the injection they were directly provided with food and the drinking water was replaced with a 5% glucose solution for a period of 24 hours to reduce the shock of the treatment. Palaloxan [1]. After 72 hours, their blood

sugar was checked using an Accu-Chek blood sugar test device of German origin to confirm that they had developed diabetes mellitus.

2.3.2. Division and Distribution of Laboratory Animals

It was confirmed that the animals had diabetes after examining the fasting blood glucose, which was 150–200 mg/dl for all the injected rats.The rats were kept in nine plastic cages with metal mesh covers and dimensions of 60, 30 and 30 cm; the cages were cleaned and sterilized with a 70% ethanol solution, and the floor was covered with sawdust that was changed every two days.It contains five infected rats in addition to the first cage in which the five healthy rats were placed as the control group. which was fed on the standard diet according to what was mentioned in [21], consisting of 35% wheat, 34% yellow corn, 20% soybeans, 10% animal protein, and 1% powdered milk, and a period of lighting of 12 hours and darkness of 12 hours. And the temperature was set at 24±2 degrees Celsius, the treatments were numbered in each cage, water was provided continuously, and they were fed the diet assigned to each treatment for the duration of the experiment, which lasted 28 days after the infection was confirmed. And they were followed up continuously under my supervision as well as that of the specialized people in the center until the end of the experiment.

For each group that was dosed orally, it differed from the other in proportions, in addition to filtered water for a period of 28 days, as the totals and ratios were as follows:

- First treatment (T1): healthy control group This group included five healthy rats.
- The second treatment (T2): a group whose five animals were infected with induced diabetes with alloxan at a concentration of 100 mg/kg of body weight without any treatment [19].
- The third treatment (T3): a group whose five animals were infected with induced diabetes with alloxan at a concentration of 100 mg/kg of body weight and orally dosed with chemotherapy (metformin) at a concentration of 50 mg/kg of body weight [22].
- The fourth treatment (T4): a group of five animals infected with alloxan at a concentration of 100 mg/kg body weight and given curcumin extract at a concentration of 50 mg/kg body weight orally [23].
- The fifth treatment (T5): a group whose five animals were infected with diabetes mellitus caused by alloxan at a concentration of 100 mg/kg of body weight and dosed orally with an extract (curcumin) at a concentration of 100 mg/kg of body weight [24].
- The sixth treatment (T6): a group whose five animals were infected with induced diabetes with alloxan at a concentration of 100 mg/kg of body weight and dosed orally with (origanum majorana) extract at a concentration of 50 mg/kg of body weight. [25].
- The seventh treatment (T7): a group whose five animals were infected with diabetes mellitus caused by alloxan at a concentration of 100 mg/kg of body weight and dosed orally with (origanum majorana) extract at a concentration of 100 mg/kg of body weight [26].
- The eighth treatment (T8): a group whose five animals were infected with diabetes mellitus caused by alloxan at a concentration of 100 mg/kg of body weight and dosed orally with an extract (vitex aguns-castus) at a concentration of 50 mg/kg of body weight [27].
- The ninth treatment (T9): a group whose five animals were infected with diabetes mellitus induced by alloxan at a concentration of 100 mg/kg of body weight and dosed orally with an extract (vitex aguns-castus) at a concentration of 100 mg/kg of body weight [28].

2.3.3. Collect Blood Samples

After the end of the 28-day experiment period, the animals were fasted for 10 hours, and then blood samples were drawn from them by cardiac puncture in an amount of (0.5–5) ml using insulin syringes with a capacity of 1 ml and placed in two sets of test tubes, one with (EDTA) ethyl diamine tetraacetic acid to prevent blood clotting and the other without, and centrifuged at high speed (at 3000 rpm) for 15 minutes to isolate blood serum. After that, it was placed in special binders and preserved by freezing at a temperature of -20 °C until it was used in conducting the necessary blood tests [29].

2.3.4. Complete Blood Cell (CBC)
CBC blood imaging tests were performed by a Sysmex device of Japanese origin, which is an analyzer device with an automated multiscale system and designed for diagnostic tests in clinical laboratories. 0.5 mL of blood was drawn and placed in tubes containing (EDTA) to prevent blood clotting for complete blood image analysis. The tube was shaken well (slowly) and then placed in the place designated for it in the device for the purpose of completing the analysis process. As the device withdraws a quantity of blood and analyzes it, the data is processed by the computer connected to the device. Criteria for blood images included white blood cells (WBC), red blood cells (RBC), volume of packed blood cells (PCV), hemoglobin HGB, mean hemoglobin MCH, and mean concentration of hemoglobin MCHC [29].

2.3.5. Determination of Serum Glucose
The concentration of glucose in the blood serum was measured using the enzymatic method [30], which included the use of the analysis kit manufactured by the German company Roche.

2.3.6. Determination of Serum Total Protein Concentration
The concentration of total protein in blood serum was measured using a ready-made assay kit from the German-made Roche company.

2.3.7. Determination of Serum Albumin Concentration
The concentration of albumin in the blood serum was measured using a ready-made assay kit from the German-made Roche company.

2.3.8. Determination of Serum Globulin Concentration
Blood globulin was estimated according to the following equation[29].

$$\text{Globulin concentration} = \text{Total protein} - \text{Albumin}$$

2.4. Statistical Analysis
The results were analyzed statistically using SAS 2010, according to a one-way analysis of variance. The mean of the coefficients was tested using the Duncun multi-rang test at a significant level (0.05) to determine the significant differences between the totals.

3. Results and Discussion

3.1. Effect on Complete Blood Picture Parameters
Given the vital physiological role that blood plays in the body's overall metabolism, some critical tests were performed on blood images. The results of the statistical analysis shown in Table 1, represented by PCV, RBC, and WBC, showed that there were significant differences (at $P \leq 0.05$) in the characteristics of white blood cells (WBC). A significant increase was observed in the second treatment (with diabetes without treatment), as the arithmetic mean of the WBC was (13,350) 10^3cells/mm^3 compared to the rest of the experimental treatments, and the rest of the treatments were within the normal range. T3, T7, T6, T4, T1, T9, T8, and T5 values were recorded at (10,500, 10.450, 10.250, 9.450, 9.050, 9.000, 8.750 and 8.650) 10^3cells/mm^3, respectively. As we have shown, the development of diabetes mellitus in mice and rabbits is accompanied by an increase in the number of white blood cells in general in all treatments treated with alloxan compared to healthy treatments. It denotes an increase in the total number of white blood cells [32]. As for the T3, metformin restores the antioxidant activity that led to a decrease in white blood cells and reduces the oxidative stress caused by alloxan, which is one of the most important causes of pancreatic damage [33]. The reason for the decrease in white blood cells in T4 and T5 treatment is because one of the most important properties of curcumin is that it is considered an anti-inflammatory by inhibiting the induction of COX-2, which is a group within NSAIDs targeting the enzyme cyclo-oxygenase. This enzyme is responsible for pain, inflammation, and the production of cytokines that increase the number of cells. and activate it [34]. Also, the presence of EBCP in the origanum majorana plant, which is an anti-inflammatory substance

even at low doses, is responsible for the decrease in white blood cells in T6 and T7 treatments [35]. The results showed that in treatments T8 and T9, giving the extract of the leaves of vitex aguns-castus led to a significant decrease in the total number of white blood cells compared to the treatment with T2 for the treatment of free radicals [36]. The results also showed that there were no significant differences in the characteristics of RBC red blood cells between the experimental treatments, and they were T5, T8, T4, T3, T7, T9, T2, T1, and T6, whose values were (6.940, 6.635, 6.390, 6.365, 6.270, 6.220, 6.105, 6.095, and 6.030) 10^3cells/mm^3, respectively. The results of the statistical analysis also showed that there were no significant differences in the characteristic of the size of the PVC-stacked blood cells between the experimental treatments, and they were T5, T4, T9, T8, T1, T2, T7, T6, and T3 whose values were (39.600, 37.750, 37.550, 36.600, 36.600, 36.400, 36.100, 36.100 and 39.050) percent, respectively.

Table 1. Effect of various medicinal plants and chemicals on the characteristics of white blood cells (WBC 10^3cells/mm^3, red blood cells RBC 10^3cells/mm^3 and PCV stacked blood cell volume %) (means ± standard error) of diabetic female rats made with alloxan.

Characteristic/ Treatment	WBC	RBC	PCV
T1	9.050±0.150 C	6.095±0.005 A	36.900±0.600 A
T2	13.350±0.450 A	6.105±0.035 A	36.600±1.500 A
T3	10.500±0.400 B	6.365±0.085 A	36.100±0.500 A
T4	9.450±0.250 BC	6.390±0.610 A	39.050±1.350 A
T5	8.650±0.350 C	6.940±0.230 A	39.600±0.700 A
T6	10.250±0.550 B	6.030±0.390 A	36.100±1.100 A
T7	10.450±0.050 B	6.270±0.130 A	36.400±0.400 A
T8	8.750±0.150 C	6.635±0.525 A	37.550±2.250 A
T9	9.000±0.300 C	6.220±0.480 A	37.750±2.750 A

* The different capital letters within one column indicate that there are significant differences ($p \leq 0.05$) between the treatments.

* Similar capital letters within one column indicate that there are no significant differences ($p \geq 0.05$) between treatments.

T1: control.

T2: She has diabetes.

T3: She has diabetes and Metformin, 50 mg/kg of body weight.

T4: She has diabetes and Curcumin 50 mg/kg of body weight.

T5: She has diabetes and Curcumin 100 mg/kg of body weight.

T6: She has diabetes and Origanum majorana of 50 mg/kg of body weight.

T7: She has diabetes and Origanum majorana 100 mg/kg of body weight.

T8: She has diabetes and Vitex aguns-castus 50 mg/kg of body weight.

T9: She has diabetes and Vitex aguns-castus 100 mg/kg of body weight.

Table 2 shows that there were significant differences in hemoglobin characteristics, with the T5 outperforming the T3 treatment, the arithmetic mean of HGB was (13.750 and 12.350) g/100 ml of blood, respectively, and there were no significant differences between treatment T5, T1, T2, T4, T6, T7, T8, and T9, as well as between treatment T3, T1, T2, T4, T6, T7, T8, and T9, and their values were (12.625, 12.450, 13.650, 12.900, 12.700, 13.350, and 12.700) g/100 ml of blood, respectively. For the T5 the reason for curcumin is that it inhibits the synthesis of hepcidin (an iron-regulating

hormone), which leads to a decrease in hemoglobin and reduces ferritin levels in the liver, so it has the ability to modify systemic iron balance in the body [37]. In the T3, hemoglobin concentration decreased in the metformin-treated groups despite blood sugar improvement. This means that metformin causes independent anemia in diabetic patients [38]. The results of the statistical analysis also showed that there were no significant differences in the mean characteristic of hemoglobin (MCH) between the experimental treatments, and they were T1, T2, T3, T4, T5, T6, T7, T8, and T9, whose values were (20.650, 21.200, 19.450, 21.450, 19.800, 21.400, 20.200, 20.200, and 20.500) pico grams, respectively. While the results of the statistical analysis showed that there were significant differences in the characteristic of the average concentration of hemoglobin MCHC, treatment T8 was superior to treatments T3 and T2, and the arithmetic mean of MCHC was (355.500, 342.500, and 339,000) grams per deciliter, respectively. No significant differences were observed between treatment T8, T1, T4, T5, T6, T7, and T9 values were (343.000, 348.500, 345.500, 350.000, 352.000, and 355,000) grams per deciliter, respectively. The T8 treatment showed a significant increase in the amount of hemoglobin and the average concentration of hemoglobin compared with the control T1 treatment and the T2 treatment, and this means that the extract of Vitex aguns-castus reduced the harmful effect of the active types of oxygen and protected the pellets from oxidative damage because the extract of Maryam leaves contains flavonoids that act as antioxidants [39].

Table 2. The effect of various medicinal plants and chemicals on the characteristics of hemoglobin HGB (g/100 ml blood), average hemoglobin MCH (pico grams), and average concentration of hemoglobin MCHC (g/deciliter) (means ± standard error) of female rats with alloxan-induced diabetes.

Characteristic/ Treatment	HGB	MCH	MCHC
T1	12.625±0.025 AB	20.650±0.050 A	343.000±3.000 ABC
T2	12.450±0.050 AB	21.200±0.500 A	339.000±4.000 C
T3	12.350±0.050 B	19.450±0.150 A	342.500±2.500 BC
T4	13.650±0.450 AB	21.450±1.350 A	348.500±0.500 ABC
T5	13.750±0.650 A	19.800±0.300 A	354.500±2.500 AB
T6	12.900±0.300 AB	21.400±0.900 A	350.000±5.000 ABC
T7	12.700±0.400 AB	20.200±0.200 A	352.000±3.000 AB
T8	13.350±0.550 AB	20.200±0.700 A	355.500±5.500 A
T9	12.700±0.400 AB	20.500±0.900 A	355.000±4.000 AB

* The different capital letters within one column indicate that there are significant differences ($p \leq 0.05$) between the treatments.

* Similar capital letters within one column indicate that there are no significant differences ($p \geq 0.05$) between treatments.

T1: control.

T2: She has diabetes.

T3: She has diabetes and Metformin, 50 mg/kg of body weight.

T4: She has diabetes and Curcumin 50 mg/kg of body weight.

T5: She has diabetes and Curcumin 100 mg/kg of body weight.

T6: She has diabetes and Origanum majorana of 50 mg/kg of body weight.

T7: She has diabetes and Origanum majorana 100 mg/kg of body weight.

T8: She has diabetes and Vitex aguns-castus 50 mg/kg of body weight.

T9: She has diabetes and Vitex aguns-castus 100 mg/kg of body weight.

3.2. Effect on Complete Blood Biochemistry

The results of the statistical analysis shown in Table (3) showed that there were significant differences in the characteristic of glucose, as the T2 recorded the highest value compared to the rest of the treatments, and the arithmetic mean was 265.460 mg/deciliter, while the T1 treatment (control) recorded the lowest the value of 105.995 mg/dl and the values for the rest of the treatments were T3, T4, T5, T6, T7, T8, and T9, (114.540, 156.025, 140.050, 126.065, 123.150, 154.485, and 147.735) mg/dl, respectively. The mouse drug Alloxan attacked pancreatic C-cells, causing a large amount of free radicals to accumulate and become toxic, eventually destroying the pancreatic beta cells responsible for insulin production. It may also have caused the development of insulin resistance. Insulin resistance and disruption of the functions of cellular receptors for insulin stop the process of receiving cells for glucose and activate the processes of glycogenolysis and the formation of glucose from non-carbohydrate sources [40]. The anti-diabetic effect of curcumin appears to be mediated through stimulation of the pancreas to produce and secrete insulin, interference with dietary glucose absorption, the insulin-sparing action of the bioactive compound component, and its antioxidant and anti-inflammatory properties [24]. Curcumin may reduce the serum glucose level of mice with early stage diabetes, raise the level of insulin and hepatic glycogen, and relieve pancreatic pain caused by diabetes [41]. In their in vitro study[42] they found an increase in glucose uptake and its fixation by skeletal muscle cells and fat cells, as well as improved function of beta cells in the pancreas, following treatment with curcumin. A research study [43] showed the effectiveness of curcumin in lowering blood glucose levels, and the reason here is due to the nature of the active components contained in the plant, such as flavones, glycosidic compounds, catechins, and phenols, and through its activation of pancreatic cells in stimulating insulin production, it has beneficial effects as well as reduces complications of diabetes. Type 2 diabetes. Also, there was a decrease in the level of glucose in the rats treated with the aqueous extract of Vitex aguns-castus, and this is due to the nature of the active ingredients and their concentrations that this plant possesses, as the leaves of the Vitex aguns-castus contain flavonoids, alkaloids, and glycosidic compounds that act as antioxidants and reduce the level of glucose [39]. The results of Table (3) also showed the presence of significant differences in the total protein characteristic, as a decrease was observed in the T2 compared with the rest of the treatments, as its values were (4.300, 7.500, 7.250, 6.850, 7.000, 7.000, 7.050, 7.100 and 7.250) g/dl, respectively. The reason for this decrease in the concentration of total protein in the blood serum of diabetic female rats may be due to exposure to oxidative stress due to the disease, leading to a decrease in the use of glucose as an energy source as a result of a decrease in insulin secretion and resorting to the use of other sources (fat and protein) as an alternative for energy, and then the catabolism of acids will increase amino acids for the purpose of obtaining the energy needed for the living body, and thus increases the process of formation of glucose from non-carbohydrate sources; or perhaps the reason for this decrease is due to complications that occur to the kidneys due to diabetes, which leads to a state of diabetic nephropathy that causes a loss of total proteins in urine [44]. It was also shown through the results of the current study that there was a significant increase in infected treatments and those treated with medicinal plants. This increase in the concentration of total protein in the blood serum can be explained for several reasons, such as the effectiveness of the antioxidant plant components such as phenols, flavones, and glycosides, which work to remove free radicals and prevent the oxidation of proteins [45].

Likewise, phenolic compounds stimulate the process of insulin secretion from pancreatic beta cells, so they stop the building of glucose from amino acids (gluconeogenesis). Also, multiple phenolic compounds reduce oxidative damage and its effects on cells and renal glomeruli, which reduces the filtration of proteins from the blood and their excretion with urine [46]. When female rats were treated with the aqueous extract of Vitex aguns-castus, a significant increase was found in the level of total proteins compared with T2. The reason is due to the role of the watery extract of Vitex aguns-castus in the process of building various proteins, especially liver proteins [47]. The results of the current study shown in the same table in the protein concentration showed that there were no significant differences between the experimental treatments, as their values were (4.350, 3.000, 4.050, 3.550, 3.650, 3.750,

3.750, 3.900, and 3.900) g/dl, respectively. The measurable decrease in serum albumin concentration of T2 infected patients without treatment could be due to the body using it as an antioxidant in the blood, or it could be due to inhibition of the activity of the albumin builder in the liver, in addition to stimulating the conversion of amino acids into carbohydrates. The decrease in albumin is one of the most severe clinical signs of liver disease and is caused by the obstruction of albumin synthesis caused by liver disease or the cause of glomerulopathy, or the decrease can be caused by free radicals resulting from oxidative stress caused by diabetes, leading to diabetic nephropathy and an increase in the amount of albumin during the process of filtration from the blood to the urine through the glomeruli [44].

Curcumin also possesses antioxidant, antimicrobial, anti-inflammatory, and hypoglycemic properties [48]. The reason for the increase in albumin in the treatments treated with the aqueous extract of the leaves of Vitex aguns-castus for the two doses is due to the effect of oxidative stress and free radicals resulting from hydrogen peroxide, which led to a disorder in the liver and kidneys, which called for the action of antioxidants in the aqueous extract of the plant and albumin is the result of the protective effect of the albumin molecule because it contains the ionic and hydrophobic character, as this characteristic gives the albumin molecule an antioxidant character through its association with the metal elements that are charged with a positive charge and that participate in the occurrence of oxidation in their various interactions when they are free and because it is widespread in the blood plasma, which is subject to continuous oxidative stress, the quantitative effect of the albumin molecule may play its role in being a good antioxidant [47]. The results of table (3) showed that there were significant differences in the characteristics of globulin, as a significant decrease was observed in the second treatment with diabetes by alloxan compared with the rest of the treatments, as its values reached (1.300, 3.150, 3.200, 3.300, 3.350, 3.250, 3.300, 3.200 and 3.350) g/dl, respectively. The cause of this decrease is attributed to the occurrence of malignancy in the glomeruli of the kidneys, as the deficiency of globulin is one of the most important clinical signs of liver disease, as the percentage of globulin decreases in the case of glomerulopathy due to albumin loss, or in the case of severe enteritis or inflammation of the intestinal lymphatic channels [49].

Table 3. Shows the effect of herbs and chemicals on glucose (mg/dl), total protein (g/dl), protein concentration (g/dl), and globulin (g/dl) (means ± standard error) of female rats with alloxan-induced diabetes.

Characteristic/ Treatment	FBS	T . protein	ALB	GLO
T1	105.995±1.605 E	7.500±0.200 A	4.350±0.250 A	3.150±0.050 A
T2	265.460±7.340 A	4.300±0.100 B	3.000±0.100 A	1.300±0.200 B
T3	114.540±4.430 DE	7.250±0.550 A	4.050±0.650 A	3.200±0.100 A
T4	156.025±2.725 B	6.850±0.150 A	3.550±0.050 A	3.300±0.100 A
T5	140.050±2.750 C	7.000±0.200 A	3.650±0.050 A	3.350±0.150 A
T6	126.065±3.065 D	7.000±0.200 A	3.750±0.450 A	3.250±0.250 A
T7	123.150±2.250 D	7.050±0.150 A	3.750±0.250 A	3.300±0.100 A
T8	154.485±4.485 B	7.100±0.200 A	3.900±0.800 A	3.200±0.600 A
T9	147.735±3.635 BC	7.250±0.050 A	3.900±0.200 A	3.350±0.250 A

* The different capital letters within one column indicate that there are significant differences ($p \leq 0.05$) between the treatments.

* Similar capital letters within one column indicate that there are no significant differences ($p \geq 0.05$) between treatments.
T1: control.
T2: She has diabetes.
T3: She has diabetes and Metformin, 50 mg/kg of body weight.
T4: She has diabetes and Curcumin 50 mg/kg of body weight.
T5: She has diabetes and Curcumin 100 mg/kg of body weight.
T6: She has diabetes and Origanum majorana of 50 mg/kg of body weight.
T7: She has diabetes and Origanum majorana 100 mg/kg of body weight.
T8: She has diabetes and Vitex aguns-castus 50 mg/kg of body weight.
T9: She has diabetes and Vitex aguns-castus 100 mg/kg of body weight.

Conclusion

All treatments had significantly lower WBC and FBS than T2 (diabetics without treatment). T2 reduced the globulin characteristic more than the other therapies. T5 outperformed T3 for hemoglobin. T8 outperformed T2 and T3 in MCHC, but not in RBC, PCV, MCH, total protein, or ALB. The current investigation shows that aqueous extracts improve blood imaging and biochemistry in female rats.

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Effect of Feed Restriction with Addition Plantain Herb on Productive Performance and Carcass Characteristics of Awassi Lambs

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Abstract. The current study aimed to find out the effect of restriction feeding with or without the addition of plantain herb on the productive performance and carcass characteristics of Awassi lambs, using 24 Awassi lambs' weight 22.18 ± 0.89 kg and ages ranged between 3.5-4.5 months. It was distributed into four groups, T1 control lambs were fed ad libitum, T2: restriction 15%, T3: ad libitum with plantain herb, T4: restriction 15% with plantain herb. The results indicated that feeding the lambs on experimental diets did not lead to significant differences in the final weight, which ranged between 36.91-39.50 kg, total weight gain which amounted to 14.75-17.16 kg, and daily weight gain which amounted to 0.163-0.190 kg, respectively. hot carcass weight increased significantly $p \leq 0.05$ in T1 (22.55 kg) compared to the other groups (21.56, 20.06, 20.45 kg, respectively). A significant increase in muscle percentage was observed, offset by a significant decrease in the thickness of subcutaneous fat ($p \leq 0.05$) in T3 compared to the other treatments. The blood results showed that the total protein concentration increased significantly ($p \leq 0.05$) for T3 and T4 (7.65, 7.13) g/100 ml compared to the T1, T2 (6.89 and 6.87) g/100 ml, respectively. While the results showed a significant decrease $p \leq 0.05$ for the concentration of urea and cholesterol 30.61, 87.03 mg/100 ml when fed plantain herb compared to the control treatment and those fed ad libitum 38.67 and 98.28 mg/100 ml, respectively.

Keywords. Feed restriction, Plantain herb, Productive performance, Awassi lambs.

1. Introduction

Ruminant breeding is one of the main pillars for achieving food and economic security for many countries of the world. It is developed quantitatively or qualitatively depending on the increasing demand for meat, by using alternatives to improve production performance and carcass characteristics. This can be achieved by adopting modern technologies that adapt to local conditions of production [1]. as a result, different nutritional strategies were used to reduce feeding costs without affecting the quality of the final product. one of these strategies is food restriction with the aim of increasing compensatory growth [2]. In this regard the effect of compensatory growth on body composition was studied by many researchers and the results were conflicting, as some studies indicated an increase in body fat content others indicated an increase in muscle tissue while other studies aimed at reducing the

actual costs of breeding [3-6]. On the other hand, and in recent times, interest in the use of herbs and medicinal plants in feeding ruminant animals has increased in order to increase productive efficiency. one of these herbs is the plantago Lanceolata. as this herb is of good nutritional importance because of its many health and medical benefits, including improving the physiological condition of the animal, reducing the need for growth stimuli and antibiotics [7]. In addition, it contains many antioxidants, represented by (Acteoside, Aucubin and Catalpol), which in turn improves production performance and induces heat stress conditions by reducing the proportion of free radicals, which is one of the main obstacles or limitations affecting production [8].In light of this many studies showed that the inclusion of plantain herb in sheep feeding led to an increase in weight rate, an improvement in the efficiency of feed conversion, an increase in the digestion of nutrients, as well as a better balance of nitrogen and an increase in its synthesis in the rumen [9]. Therefore, the current study aimed to determine the feed restriction to reduce the cost of feeding and add plantain herb to increase the efficiency of utilization of food and its impact on the productive performance and carcass characteristics of Awassi sheep.

2. Material and Method

This study was conducted in the Animal Production Department of the College of Agriculture and Forestry - University of Mosul. this is to find out the effect of dietary restriction with or without plantain herb on the productive performance and carcass characteristics of Awassi lambs. In this study, 24 Awassi lambs were used, with an average Initial weight 22.18 ± 0.89 kg and age ranging between 3.5-4.5 months. It was distributed into four groups, first was control its lambs were fed on a standard diet (ad libitum), the second fed restriction diet at 15% of the control treatment, the third was fed ad libitum with the addition of plantain herb at a rate of 3%, while the lambs in the fourth treatment were fed restriction 15% and add 3% plantain herb. The lambs were fed on experimental diets gradually for a period of 15 days as a preparatory period. During the 90-day trial period, the free feeding system was followed for the first and third treatments, as the remaining feed was collected daily and weighed to calculate the actual intake, while the specific feeding system was followed in the second and fourth treatments as well. provide water and mineral blocks continuously in front of the animals throughout the study period. Table (1) shows the components and chemical composition of the experimental diets used in the study during the study period and in the middle and at the end of the period, the blood sample was withdrawn from the jugular vein using a plastic syringe with a capacity of 10 ml, and the blood serum was separated by centrifugation (3000 revolutions / min) for 15 minutes and the samples were kept under freezing (20 °C) until analysis. blood samples were analyzed using a ready-made analysis kit (Biolabo) of French origin, samples were read by a spectrophotometer (Biotech Engineering Management Co.LTD.uk). the end of the study period, the lambs were weighed to establish the final weights before slaughter, and the feed was cut off from the lambs for a period of 12 hours, and then 3 lambs were slaughtered from each treatment. kidney [10]. After that, the carcass was separated into two equal halves, and the ribs (9-10-11) were taken from the left half to conduct a physical inventory [11]. The eye rib aera and the thickness of the subcutaneous fat between ribs 12 and 13 were also measured, as reported by [12]. The statistical analysis was conducted using a complete randomized design (CRD) for an experiment with two factors, first (effect of ad libitum and restriction) second (effect adding plantain herb). The data were analyzed statistically using SAS software [13], and significance between the means was tested using Duncan's multiple range test [14].

Table 1. Chemical components and composition of the experimental diets.

Ingredients	Experimental diets			
	T1	T2	T3	T4
Crushed barley	69	69	68	68
Wheat bran	18	18	17.5	17.5
Plantain herb	---	---	3	3
Soyabean meal	5	5	5	5
Wheat straw	5.5	5.5	4	4
Urea	0.5	0.5	0.5	0.5
salt	1	1	1	1
Calcium carbonate	1	1	1	1

Ingredients	Experimental diets			
	T1	T2	T3	T4
Chemical composition %				
Dry matter	91.39	91.39	92.34	92.34
Crude protein	14.14	14.14	14.34	14.39
Metabolism energy, kcal / kg	2579	2579	2495	2495

Chemical composition was laboratory determined according to AOAC [15].

3. Result and Discussion

Table (2) shows that the amount of dry matter was low in the T2 and T4 treatments, whose lambs were fed on restriction diet 15% and reached 896.82 and 896.34 g/day compared to 1257 and 1265 g/day respectively, for the amount of dry matter ingested in T1 and T3 were fed ad libitum. The same was the case for the protein intake which amounted to 126.81 and 125.09 g/day for the lambs of the T2 and T4 groups compared to the amount of protein intake for the lambs of T1 and T3 treatments which amounted to 177.73 and 182.03 g/day respectively. In addition to the decrease in the amount of energy intake for the lambs of specific feeding, which amounted to 2312.92 and 2236.36 kcal / kg, compared to ad libitum fed lambs which amounted to 3241.80 and 3156.17 kcal / kg respectively. On the other hand, the restriction feed and the addition of plantain herb to the lambs of the fourth treatment improved the efficiency of feed conversion 6.37 kg feed intake / kg weight gain compared to other treatments, which amounted to 8.23, 7.00 and 8.66 kg feed intake / kg weight gain, respectively. The results of the current study agreed with what was reported by [16-18], that the amount of dry matter ingested by lambs fed restriction diets decreased, and contrary to what was found by [19], that restricted feeding of lambs led to an increase in the efficiency of feed conversion. In addition, the results agreed with [20,21], who proved that feeding lambs on diets containing plantain herb led to a significant increase in the amount of dry matter ingested. the improvement in the efficiency of feed conversion for lambs of the fourth treatment, fed on restricted diet with plantain herb may be due to the presence of sorbitol in the herb which is an alcoholic sugar containing six carbon atoms and is derived from glucose and works to change the fermentation pattern in the rumen by increasing propionic content and reducing steak [22].

Table 2. Effect of feeding system with or without plantain herb on productive performance of lambs.

Treatment/ Characteristics	T1	T2	T3	T4
Dry matter intake gm/day	1257	896.82	1265	896.34
Protein intake gm/day	177.73	126.81	182.03	125.09
Energy intake Kcal/day	3241.80	2312.92	3156.17	2236.36
Feed Efficiency	8.23	7.00	8.66	6.37
Initial weight kg	22.16±1.19 a	22.16±0.88 a	22.25±0.72 a	22.16±0.79 a
Final weight kg	39.50±0.77 a	36.91±1.35 a	38.42±0.74 a	38.20±1.04 a
Total weight gain kg	17.16±1.18 a	14.75±0.77 a	16.17±0.77 a	16.04±1.34 a
Average daily gain gm/day	0.190±0.01 a	0.163±0.01 a	0.179±0.00 a	0.177±0.01 a

T1: ad libitum, T2: restriction 15%, T3: ad libitum with plantain herb, T4: restriction 15% with plantain herb.

The productive performance of lambs, the results in Table (2) indicate that there are no significant differences between the treatments in the average initial weight, as well as the average final weight of the four groups 39.50, 36.91, 38.42,38.20 kg, and total weight gain 17.16, 14.75, 16.17, 16. 04 kg and daily 0.190, 0.163, 0.179, and 0.177 kg/day respectively. The results of the current study agreed with [17,23], who indicated that determining the level of nutrition did not have a significant effect on the productive performance of lambs. It does not agree with the results of [24-27]. At different levels it led to a decrease in the productive performance of the lambs, which is represented by the final weight and the rate of daily weight gain. The improvement of the productive performance of the lambs of the two food rationing treatments may be due to the improvement in the efficiency of feed conversion as a result of reducing the mass of food filling the rumen to 85% and enabling its digestion microbially and mechanically in an adequate manner, which improved the efficiency of its food conversion and improved its growth characteristics compared to the lambs of the control treatment. In addition, the results of the current study were consistent with what was reported by [28,29], that feeding lambs at

different levels of plantain herb did not lead to significant differences in productive performance. On the other hand, the results were contrary to what was found by [20,21,30], that feeding lambs at different levels of plantain herb led to significant differences in the productive performance of lambs. The improvement in the final weight of the lambs of the T4 fed on restricted diet 15%, and addition of plantain herb may be due to the presence of the herb which in turn leads to an improvement in the rumen environment and the digestion of nutrients by increasing the activity and effectiveness of fiber-decomposing bacteria and inhibiting the growth of methane-producing bacteria which in turn leads to death. To reduce energy loss through the formation of methane gas, as well as the presence of biologically active compounds in the herb represented by (Polyphenolic and Acteoside) which have a positive role in energy metabolism by reducing the numbers of methanogenic bacteria [31]. The results indicated in Table (3) that the weight at slaughter increased mathematically in the first and second treatments 42.87 and 41.37 kg compared to the third and fourth treatments 39.75 and 39.56 kg. The improvement of the productive performance of the lambs of the second and fourth treatments fed restricted diet with or without plantain herb may be due to the improvement in the efficiency of feed conversion as it was the best in the above two treatments amounting to 7.00, 6.37 kg feed / kg weight gain, compared to the first and third treatments, which amounted to 8.23 and 8. 66 kg feed / kg weight gain, respectively. In addition, the results indicate that the empty body weight T1 was significantly high ($p \leq 0.05$) 39.22 kg compared to the lambs of the T3, T4 which amounted 37.37 and 37.38 kg, respectively. The weight hot carcass decreased significantly ($p \leq 0.05$) in the three treatments and reached 21.56, 20.06, 20.45 kg compared to the control treatment which was 22.55 kg.

Table 3. Effect of feeding system with or without plantain herb on carcass characteristics of lambs.

Treatment/ Characteristics	T1	T2	T3	T4
Slaughter weight kg	42.87±0.87 a	41.37±1.23 a	39.75±0.66 a	39.56±1.33 a
Empty body weight kg	39.22±0.30 a	38.59±0.95ab	37.37±0.62 b	37.38±1.21 b
Hot Carcass weight kg	22.55±0.12 a	21.56±0.75 b	20.06±0.54 b	20.45±0.57 b
% Dressing percentage	52.64±0.98 a	50.96±1.93 a	50.44±0.61 a	51.74±0.62 a
Eye muscle area Cm^2	15.83±0.88 a	15.75±0.62 a	17.25±0.24 a	16.50±0.28 a
Thickness subcutaneous fat/mm	9.16±1.58 ab	10.50±0.76 a	8.00±0.57 ab	7.00±1.15 b
% Total fat percentage	23.30±1.60 a	24.91±1.00 a	24.49±1.11 a	24.39±0.50 a

T1: ad libitum, T2: restriction 15%, T3: ad libitum with plantain herb, T4: restriction 15% with plantain herb.
* a,b, Mean values within a row with different superscripts differed ($P \leq 0.05$).

No significant differences were observed between the groups in the dressing percentage on the basis of weight at slaughter, which amounted to 52.64, 50.96, 50.44,51.74%, as well as the eye muscle area which amounted to 15.83,15.75,17.25,16.50 cm^2 respectively. Although no significant differences were recorded in the dressing percentage and eye muscle area, the thickness of subcutaneous fat was the least significant ($p \leq 0.05$) for the lambs of T4 compared to the other treatments, and reached 9.16, 10.50, 8.00, 7.00 mm, respectively, as it is usually there is a negative correlation between the degree of body muscle and the thickness of subcutaneous fat [32]. Also, the differences were not significant in the percentage of total fat, which represents the ratio of the total fat of the carcass to the weight of the carcass, and reached 23.30, 24.91, 24.49, 24.39%, respectively.

The results agreed with what was reported by [25,26], in their study that restriction feeding of lambs led to a significant decrease in the weight of the hot carcass. While it did not agree with [33,34], who indicated that feeding lambs on plantain herb did not lead to significant differences in the weight of the hot carcass. The presence of the active substance Acteoside in the plantain herb leads to a change in the expression of leptin towards reducing the catabolism of cholesterol and accelerating the process of beta-oxidation of fatty acids, and this explains our observation of a decrease in the thickness of the subcutaneous fat of the lambs of the fourth treatment [35].

Table 4. Effect of feeding system with or without plantain herb on Physical inventory of cut ribs (9-10-11) of lambs.

Treatment/ Characteristics	T1	T2	T3	T4
% Percentage fat	27.49±2.85 a	28.72±3.69 a	24.81±0.54 a	28.96±1.02 a
% Percentage lean	49.30±2.30 c	46.45±2.50 d	55.11±0.69 a	51.86±1.63 b
% Percentage bone	23.19±0.55 ab	24.81±2.69 a	20.07±0.17 b	19.16±2.56 b

T1: ad libitum, T2: restriction 15%, T3: ad libitum with plantain herb, T4: restriction 15% with plantain herb.
* a,b, Mean values within a row with different superscripts differed (P ≤ 0.05).

Table (4) shows the results of the physical separation (9-10-11), as it turns out that the percentage of fat was insignificant among the treatments, amounting to 27.49,28.72,24.81,28.96%, respectively. The percentage of lean between the experimental groups were significantly different (p≤0.05), and the lowest lean percentage was recorded for the first and second treatments, amounting to 49.30 and 46.45%, while the highest lean percentage was recorded for the third treatment, whose lambs were fed ad libitum with plantain herb, and amounted to 55.11%, while the percentage of bone It was significantly high (p≤0.05) in the first and second treatments, reaching 23.19 and 24.81%, compared to 20.07 and 19.16% for the third and fourth treatments, respectively. It is clear from the results of the physical separation that the eye muscle area of the carcasses and the degree of muscle tissue of the carcass are positively correlated with each other with a value of 0.9 [36]. Referring to the results of the physical separation in the table above for the control treatment lambs, which were fed ad libitum this led to an increase in building their fat tissue and decreasing their lean tissue compared to the third treatment lambs, as well as the role of the plantain herb in balancing nitrogen by reducing its excretion in the urine and its reflection on increasing protein synthesis rates in the whole body [37].

It is noted from Table (5) that feeding the lambs on plantain herb in the third and fourth treatments led to a significant increase (p≤0.05) in the total protein concentration and amounted to 7.65, 7.13 g/100 ml compared to the first and second treatments and amounted to 6.89, 6.87 g/100ml, respectively. The albumin concentration was significantly higher (p≤0.05) in the second treatment was 4.83 g/100ml compared to the other groups and reached 4.56,4.41,4.39 g/100 ml, respectively. the concentration of globulin increased significantly (p≤0.05) in the third treatment whose lambs were fed plantain herb, and reached 3.24 g/100ml compared to the other treatments and reached 2.33,2.04,2.74g/100ml respectively. While the differences were not significant for the concentration of glucose in the blood which reached 63.38,64.56,65.72,64.45mg/100ml, respectively. As for the urea concentration, it was significantly low (p≤0.05) in the three treatments, reaching 35.37, 34.07, 30.61 mg/100 ml compared to the control treatment which was 38.67mg/100ml respectively. On the other hand the results in Table (5) showed that feeding lambs on plantain herb in the third and fourth treatments had a significant decrease (p≤0.05) in cholesterol concentration and reached 88.58, 87.03 mg/100ml compared to its concentration in the first and second treatments which amounted to 98.28 , 94. 84 mg /100 ml respectively, as well as a decrease in the value of triglycerides for the third and fourth treatments, whose lambs were fed ad libitum or restricted with the addition of plantain herb to reach 49.59 , 46.81 mg /100ml, which differed significantly (p≤0.05) from the first and second treatments and reached 58.11,54.75 mg/100ml respectively.

Table 5. Effect of feeding system with or without plantain herb on blood parameters of lambs.

Treatment/ Characteristics	T1	T2	T3	T4
Total protein gm/ml	6.89±0.24b	6.87±0.12b	7.65±0.08a	7.13±0.15a
Albumen gm/ml	4.56±0.08b	4.83±0.02a	4.41±0.09b	4.39±0.12b
Globulin gm/100 ml	2.33±0.21b	2.04±0.14c	3.24±0.14a	2.74±0.17b
Glucose mg/100ml	63.38±1.62a	64.56±2.22a	65.72±1.51a	64.45±0.64a
Urea mg/100 ml	38.67±0.91a	35.97±0.59b	34.07±0.79b	30.61±0.64c
Cholesterol mg/100 ml	98.28±1.93a	94.84±1.79a	88.58±1.88b	87.03±1.72b
Triglycerides mg/100ml	58.11±1.66a	54.75±0.96b	49.59±1.88c	46.81±0.70c

T1: ad libitum, T2: restriction 15%, T3: ad libitum with plantain herb, T4: restriction 15% with plantain herb.

* a,b, Mean values within a row with different superscripts differed ($P \leq 0.05$).

The results were inconsistent with what was shown by [26,38], that determining the feeding of lambs at different levels did not have a significant effect on the biochemical characteristics of blood. and they agreed with each of [21,33], as they explained that the inclusion of plantain herb in lamb diets led to a decrease in the concentrations of both blood urea, cholesterol, and triglycerides. In light of this [39], indicated in their study that the presence of the active substances (catapol and aucubin) in plantain herb prevents abnormal assimilation or formation of glycolipids by reducing oxidative stress and the formation of glycation end products, which are likely to be related to levels Low triglycerides and cholesterol. In addition, catapol is an active diuretic and increases kidney activity which leads to a decrease in the level of creatinine and urea in the blood [40]. Interestingly, it was observed through the study that the lambs that consumed diets containing the herb consumed more amounts of water compared to the lambs of the other groups.

Conclusion

This study showed that lambs with 85% restriction feed with or without plantain herb due to higher feed efficiency. In addition, morphological changes in the rumen and intestine allowed greater absorption capacity.

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The Vital Effect of Zinc, Spirulina Algae Powder and their Combination on some Physiological and Productive Traits of Lactating Iraqi Local Goats and Their Suckling Offspring

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Abstract. The late gestation and lactation period of mammals requires the support of diet supplements that have high nutritional value and medicinal properties to avoid the occurrence of metabolic and health disorders that negatively affect maternal and their suckling offspring. Therefore, this study investigates the effect of dietary supplementation of spirulina algae powder (Spirulina Platensis) and zinc (ZnSo4) and their combination on the physiological and productive parameters of Iraqi native goats. This study was conducted on 20 local Iraqi goats, 2-4 years old, divided into four groups (n=5). The 1st group was untreated and was used as a control (Co), while the 2nd group were supplemented with zinc (Zn) 50 mg/kg diet, the 3rd group with spirulina (Sp) 5 g/kg diet, and 4th group was treated with zinc and spirulina (Zn+Sp) at the same previous levels. The duration of the study was 120 days persisted from mid-gestation until early lactation. The recorded indicators were some biochemical, metabolic and reproductive hormones, oxidative state, production and milk components. The results showed that there were significant differences ($P \leq 0.05$) in otal protein, leptin level, prolactin, superoxide dismutase, malondialdehyde, milk yield, milk protein and offspring growth indicators. In conclusion, the treatment of spirulina algae powder and zinc and their combination had a positive effect on the physiological and productivity status when treated in the physiological stages (late gestation and early lactation), which was reflected in the improved growth performance of suckling kids.

Keywords. Spirulina, Zinc, Insulin, Leptin, Prolactin, Milk, Goat.

1. Introduction

The lactation stage is the most energy-expensive period in the mammalian reproductive cycle, average daily caloric intake can be 66-200% greater, and peak energy expenditure maybe 2.5-5 times higher in lactating females than in non-lactating females [1]. At the lactation stage, behavioral changes often coincide with increased energy demand, for example, females may dramatically increase the duration and frequency of foraging in order to balance increased energy use. In addition, to increased forage intake, females are able to gnaw at body tissues to meet the deficiency of nutrients [2]. Therefore,

ensuring a balanced diet in terms of energy, protein and other essential elements during late pregnancy and lactation is crucial to ensuring the animal's optimal long-term performance. Continuous consumption of unbalanced diets that are not consistent with the animal's productive state may lead to increased oxidative stress and inflammatory conditions in the organism, which impairs reproductive performance and milk production, and nutritional supplements may give beneficial results in counteracting the harmful effects resulting from the lack of nutrients necessary for pregnancy and lactation [3]. Microalgae are found in marine and freshwater environments and are classified as eukaryotes or prokaryotic cyanobacteria, and many species of algae have been used as a common feed source for field animals and aquatics for many years [4]. The first experiments on the use of unicellular microalgae species as fodder for ruminant animals were conducted less than twenty years ago [5], which occurred only in the past decade on sheep [6] and cattle [7]. The recent awareness of climate change has renewed interest in the use of algae in large numbers, e.g. spirulina algae, as a mitigant for enteric methane emissions of ruminants [8]. Microalgae supplements known as spirulina have been shown to enhance the reproductive performance of animals [9,10] by improving the antioxidant status of animals, including increasing the antioxidant capacity of plasma, since spirulina is rich in bioactive phytochemicals [11]. Among them are pigments such as phycocyanin, carotenoids, and tocopherols [12] Spirulina also contains high levels of bioactive long-chain fatty acids, especially linolenic and linoleic acid, whose intake during pregnancy and lactation may be beneficial for both maternal and developing fetus. The unique fatty acid properties of spirulina may also be reflected in the composition of milk and thus affect the growth and health of offspring after parturition [13].
Various studies were conducted to investigate the effect of zinc supplementation on lactating animals, and zinc was used in its organic and inorganic form, and there was an equivocal response to zinc supplementation on milk production and composition [14,15]. Zinc is considered nutritionally one of the most important trace minerals that participate in various biochemical and physiological processes supporting the body of the organism [16]. It is an important constituent of a variety of metalloenzymes and hormones and acts as a catalyst for more than one enzymatic reaction in the animal body [17,18]. It plays an important role in maintaining body growth and the health and integrity of epithelial tissues through its influence on cell division and protein synthesis [19,20]. Zinc is not stored in the body to a large extent and thus is required to be taken on a daily basis to maintain normal physiological functions [21].
Goats are one of the important ruminants for animal products all over the world. Goats have important characteristics such as their ability to adapt and survive under the harsh environmental conditions in Iraq. The number of goats in Iraq is estimated to be about 1.5 million heads [22]. Goats are raised mainly for the purpose of producing Meat and milk [23]. In contrast to other field animals, limited research has been conducted to determine or improve the reproductive efficiency of Iraqi native goats [24]. It was found that physiological stages such as pregnancy and lactation are critical periods that affect the health and metabolic status of mammals [25,26]. As the amounts of feed intake and body composition are modified in order to provide adequate amounts of nutrients for fetal growth and milk production, moreover, biochemical and hormonal variables that are important indicators of the nutritional and health conditions of animals are modified [27].

2. Materials and Methods

2.1. Animals and Treatments

This study was conducted in the field of ruminants at the College of Agriculture - Tikrit University - Iraq. 20 pregnant Iraqi local goats (gestational age ranges from 45-60 days), whose ages ranged from 2-4 years, were used. It was randomly divided into four similar groups of five animals in each group on the basis of their body weight, they were housed and fed collectively. The diet of the 1st group was untreated and served as control (co), while the diet was supplemented with zinc sulphate (Zn) at a rate of 50 mg/kg diet in 2nd group, or spirulina algae powder (Sp) at a rate of 5g /kg diet of 3rd group, and combined 50mg zinc + 5g spirulina (Zn+Sp) in 4th group. The experimental period was 120 days (ninety days prenatal and thirty days postnatal). The animals were administered zinc capsules every morning, while the spirulina algae powder was mixed and provided with concentrated feed.

Spirulina algae powder *(Spirulina platensis)* imported from Inner Mongolia of the China Republic (Etuokeqi Kangsheng Spirulina Co., Ltd). The use of inorganic zinc in the form of zinc sulphate ($ZnSo_4$) supplied by CDH, India was obtained within the country.

2.2. Blood Collection and Laboratory Analyses

Blood samples (8 ml blood/head) were collected from the goat and their offspring directly from the jugular vein on the 30th day after parturition. The blood samples were centrifuged (3000 r/min) for 15 minutes, and the serum was separated and stored (-20 °C) until use. Leptin was measured using the ELISA technique. While Insulin, Thyroxine (T4), Triiodothyronine (T3), Estrogen, Progesterone and Prolactin, were measured using the Cobas e 601 devices. Mindray BS-430 device was used to measure the biochemical parameters (Glucose, Triglycerides, Cholesterols, Total protein, Total Antioxidant Capacity (TAC), Glutathione (GSH), Superoxide Dismutase (SOD) and Malondialdehyde (MDA).

2.3. Milk Yield and Chemical Composition

The milk amount was measured in the second and fourth weeks after goats were kidding, using a sensitive electronic scale. A day before measuring the amount of milk, the offspring were separated from their maternal, and an udder of milk was emptied. The next morning, the goats were milked by manual method, and the milk amount for each goat was measured by multiplying the amount of milk collected x 2 to estimate the amount of produced daily milk [28]. The chemical composition of milk was analyzed using the Eko-milk Analyzer (Funke-Gerber, Germany) Obtained in the laboratories of the Agriculture College / University of Tikrit.

2.4. Statistical Analysis

SAS statistical software was used to analyze all data by one-way analysis of variance (ANOVA) and Duncan's multiple range test [29] to find out the differences between the means at the 5.0% level of significance [30].

3. Results and Discussion

3.1. Biochemical Parameters

The outcomes of the biochemical analysis indicated that there was a significant ($P \leq 0.05$) increase in serum Total protein (Table 1) of Sp and Zn+Sp treatments compared to a control group, while there was no significant difference between the Zn treatment and control. At the same time, we did not find any significant ($P \leq 0.05$) effects of treatments on parameters of Glucose, Triglyceride and Cholesterol levels.

Table 1. Effect of spirulina algae powder, zinc and their combination on some biochemical parameters of Iraqi local goats.

Parameters	Control	Zn	Sp	Zn + Sp
Glucose (mg/dL)	53.23 ± 3.53 a	50.97 ± 4.48 a	50.46 ± 4.01 a	49.61 ± 3.72 a
Triglyceride (mg/dL)	22.85 ± 2.02 a	22.14 ± 1.78 a	20.21 ± 1.27 a	19.73 ± 1.83 a
Cholesterol (mg/dL)	60.92 ± 2.15 a	61.16 ± 2.59 a	59.76 ± 2.77 a	60.57 ± 2.69 a
Total protein (g/L)	64.28 ± 2.92 b	67.11 ± 1.12 ab	67.86 ± 1.19 a	70.32 ± 2.94 a

The values represent the mean ± standard error; Different letters within the same row are significantly differ at ($P \leq 0.05$).

The Total protein level in the blood is closely related to the nutritional status of the body, so the improvement in protein metabolism is attributed to the high level of crude protein, essential amino acids, vitamins, minerals, phospholipids, and antioxidants [31]. Or it may be attributed to the combined effect of zinc and spirulina in increasing the level of superoxide dismutase (SOD) enzyme

and reducing lipid peroxidation (Table 2) in these animals. It was found that the increase in the level of SOD enzyme and the decrease in lipid peroxidation reduced tissue deformities. and biochemical disorders associated with the manufacturing and secretory functions of the liver [32]. Prasad [33] indicated that zinc deficiency may cause abnormalities in DNA synthesis and the activity of several enzymes, so it could be that the increase in serum proteins could be due to the role of zinc in protein synthesis. This result came in agreement with the results of researchers' studies [34,35], who found that treating rats and small ruminants with spirulina algae led to an increase in the level of total protein in the blood. It also found similar effects to zinc in poultry, poultry, and swine [36,37]. On the contrary, the researchers found no significant effects of spirulina [39] or zinc [38] on serum total protein levels.

3.2. Anti/or Oxidative Stress Indicators

The results of the statistical analysis in Table 2 show that the Superoxide dismutase (SOD) enzyme and malondialdehyde (MDA) were affected ($P \leq 0.05$) by the experimental treatments, while the level of Total antioxidant capacity (TAC) and Glutathione (GSH) remained constant and was not affected by the treatments. Where the treatment Zn + Sp was superior to the Sp and Zn treatments in increasing the SOD enzyme compared to the control. For MDA, the three treatments were equal in effect compared to the control group.

Table 2. Effect of spirulina algae powder, zinc and their combination on some anti/or oxidative parameters of Iraqi local goats.

Parameters	Control	Zn	Sp	Zn + Sp
TAC (U/ml)	3.86 ± 0.53 a	3.91 ± 0.69 a	4.31 ± 0.57 a	4.12 ± 0.48 a
GSH (µmol/ml)	1.16 ± 0.24 a	1.20 ± 0.35 a	1.17 ± 0.30 a	1.14 ± 0.31 a
SOD (U/ml)	1.26 ± 0.32 c	3.46 ± 0.65 ab	2.19 ± 0.38 b	4.30 ± 0.97 a
MDA (µmol/L)	2.36 ± 0.38 a	1.17 ± 0.34 b	1.21 ± 0.36 b	0.96 ± 0.33 b

The values represent the mean ± standard error; Different letters within the same row are significantly differ at ($P \leq 0.05$).

The role of spirulina in increasing the level of SOD enzyme by containing good proportions of major and trace minerals [40]. It requires the presence of some elements such as magnesium, iron, copper and zinc to stimulate the activation and synthesis of the enzyme SOD [41]. As it binds to zinc, it is one of the structural components of the superoxide dismutase enzyme found in the cytoplasm of cells. This enzyme contains an active center of zinc and copper ions. Therefore, maintaining adequate concentrations of zinc in cell compartments is essential for the proper functioning of the antioxidant defense system, as this enzyme is the first line of defense by converting the more toxic superoxide radical into the less toxic hydrogen peroxide molecule, which can then be converted into water and oxygen by catalase enzyme [42]. The results of our study agreed with previous studies [43,44,45,46] and contradicted others [47,48]. With regard to the decrease in serum MDA concentration under the influence of zinc supplementation, it may be due to the susceptibility of zinc to bind to the metallothionein protein under normal physiological conditions. In conditions of oxidative stress, zinc is released from the metallothionein and redistributed in the cells to exert its antioxidant actions, as zinc stimulates the production of Metallothionein and is an effective hydroxyl radical scavenger [49]. As for the role of spirulina algae, it may be attributed to the effect of phycocyanin, a water-soluble protein that has a catalytic role in activating the enzyme catalase, which counteracts reactive oxygen species and scavenges free radicals, Phycocyanin also contributes to the downregulation of the enzyme Inducible nitric oxide synthase (iNOS) and thus the decrease in the production of nitrite oxide, which is converted by reactive oxygen species into peroxynitrite (NO-3) that causes oxidative stress, and thus inhibits lipid peroxidation [50]. As well as increasing of the SOD enzyme level which may have

contributed to the reduction of MDA levels. The results of this study supported the results of previous studies on the effect of spirulina algae [51] and zinc [52] in reducing lipid peroxidation indicators.

3.3. Hormonal Parameters

Table 3 illustrates that goats given Zn and Zn+Sp expressed higher levels ($P \leq 0.05$) of leptin hormone compared with the control goat, while Spirulina treatment did not show any differences compared to the control. At the same time, we also saw that the goats treated with Zn + Sp supplementation had the highest levels ($P \leq 0.05$) of prolactin compared to the control goats, while the differences were non-significant between the Zn and Sp groups compared to the control. On another hand, we did not find any differences with respect to Insulin, T4, T3, Estrogen and Progesterone between the experimental groups.

Table 3. Effect of spirulina algae powder and zinc and their combination on some hormonal parameters of Iraqi local goats.

Parameters	Control	Zn	Sp	Zn + Sp
Insulin (μIU/ml)	0.84 ± 0.25 a	0.79 ± 0.14 a	0.46 ± 0.14 a	0.65 ± 0.08 a
Leptin (pg/mL)	1108.85 ± 51.93 b	1305.89 ± 55.17 a	1079.98 ± 54.77 b	1273.77±41.43 a
Thyroxine (nmol/L)	53.42 ± 4.89 a	52.73 ± 4.20 a	52.14 ± 3.68 a	51.56 ± 4.81 a
Triiodothyronine (nmol/L)	1.46 ± 0.26 a	1.42 ± 0.14 a	1.37 ± 0.20 a	1.28 ± 0.17 a
Estrogen (pg/mL)	10.63 ± 0.79 a	10.12 ± 0.46 a	11.09 ± 0.80 a	9.27 ± 0.64 a
Progesterone (ng/mL)	2.38 ± 0.48 a	2.07 ± 0.32 a	2.21 ± 0.26 a	1.81 ± 0.37 a
Prolactin (ng/mL)	17.53 ± 0.84 b	22.03 ± 1.27 ab	19.34 ± 1.53 ab	22.72 ± 1.04 a

The values represent the mean ± standard error; Different letters within the same row are significantly differ at ($P \leq 0.05$).

Regarding the leptin hormone, there is a relationship between the lactation period and the leptin hormone, and the hormone may play an important role in the distribution of nutrients and energy in the mammary gland during lactation, and that leptin plays the role of Autocrine Paracrine in the milky tissues for the purpose of epithelial cell proliferation, differentiation and growth in the presence of prolactin [53,54]. The significant increase in the level of leptin, both in the 2nd treatment (Zn) and 4th treatment (Zn + Sp), may be due to the fact that zinc synergizes with the prolactin hormone in increasing leptin resistance, as zinc stimulates the expression of mRNA of appetite-stimulating neuropeptides, especially Neuropeptide Y (NP-Y), which in turn impedes The leptin pathway through inhibition of satiety-inducing Melanocortin 4 (MC4) receptors, which leads to leptin accumulation in the blood [55]. These results corroborated those of [56,57] who found that treatment with zinc supplementation increased leptin levels. Other than this finding, [58] did not find an effect of zinc on serum leptin levels.

As for the combined effect of zinc and spirulina on the significant increase in the prolactin (lactogenic) hormone level, It may be attributed to the role of zinc in increasing the level of the leptin hormone (Table 3), which is a strong stimulus for the secretion of the prolactin hormone, as it is known that leptin receptors are located in all parts of the hypothalamus and pituitary gland, and the stimulation takes place either in a direct way through Activation of extracellular signal-dependent kinase pathways [59]. Or the reason for the high prolactin level is due to the element selenium available in spirulina algae, as it was found that selenium increases the level of prolactin hormone by stimulating gene expression for prolactin synthesis [60]. The present result agrees with the results of previous studies [61], which found an increase in prolactin levels in rats treated with spirulina algae. And with [62],

who obtained a significant increase in the level of prolactin hormone for ewes treated with zinc in the late pregnancy period and the postpartum period.

3.4. Yield and Composition of Milk

It is clear from the data summarized in Table 4 that the experimental treatments did not affect the quantity and quality of milk goat in the 2nd-week post-kidding, while 4th-week post kidding we found a significant ($P \leq 0.05$) increase in the amount of milk production and milk protein for goats in the 3rd group (Sp) and 4th group (Sp+Zn) compared to the control group (The percentage increase is 31% and 43%, respectively). When the differences were not significant in the milk yield and milk protein of the goats of the 2nd group (Zn) compared to the control goats, at the same time we did not notice any differences in the other milk components (fat, lactose and non-fat solids).

Table 4. Effect of spirulina algae powder and zinc and their combination on yield and composition of milk after 2 and 4 weeks of goat kidding.

Parameters	Week	Control	Zn	Sp	Zn + Sp
Milk yield (g)	2 wk.	643.98 ± 55.90 a	694.13 ±59.85 a	638.24 ±65.70 a	717.44 ±69.12 a
	4 wk	778.05 ± 30.23 b	863.41 ±53.74 ab	914.13 ±46.26 a	945.46 ±34.38 a
Fat (%)	2 wk.	3.20 ± 0.36 a	3.35 ± 0.28 a	3.13 ± 0.39 a	3.27 ± 0.37 a
	4 wk	2.60 ± 0.41 a	2.95 ± 0.41 a	2.53 ± 0.54 a	2.87 ± 0.36 a
Protein (%)	2 wk.	2.94 ± 0.49 a	3.06 ± 0.47 a	3.22 ± 0.52 a	3.17 ± 0.57 a
	4 wk	3.09 ± 0.24 b	3.37 ± 0.23 ab	3.76 ± 0.26 a	3.84 ± 0.18 a
Lactose (%)	2 wk.	4.52 ± 0.19 a	4.64 ± 0.32 a	4.61 ± 0.19 a	4.78 ± 0.12 a
	4 wk	4.34 ± 0.21 a	4.83 ± 0.32 a	4.91 ± 0.22 a	4.96 ± 0.18 a
Non-fat solids (%)	2 wk.	8.26 ± 0.45 a	8.46 ± 0.46 a	8.58 ± 0.33 a	8.71 ± 0.39 a
	4 wk	8.15 ± 0.33 a	8.96 ± 0.25 a	9.49 ± 0.29 a	9.64 ± 0.34 a

The values represent the mean ± standard error; Different letters within the same row are significantly differ at ($P \leq 0.05$).

These results may be attributed to the synergistic action of zinc and spirulina in increasing the production and secretion of the hormone prolactin (Table 3), which is the main hormone stimulating the production of milk and milk protein. Or it may be due to the presence of alkaline elements and other substances in spirulina algae, which are very important in preserving the rumen environment from high acidity because the activity of microorganisms and the fermentation process are more effective in a moderate environment, and this helps to form enough volatile fatty acids necessary for the functions of organisms in the rumen. Milk production [63]. Zinc may also have a role in increasing milk production by stimulating the synthesis of proteins and regulating the proliferation, differentiation, and growth of epithelial cells of udder tissues by intervening in modulating receptor pathways for hormones responsible for udder development and milk production, such as growth hormones, estrogen, and prolactin [64]. There are many previous studies that support our current results, as Lamminen et al. [65] found an increase in milk production and protein percentage in Ayrshire cows treated with spirulina algae powder for 21 days. Mohamed et al. [62] also treated ewes with Nano-zinc 5 and 10 mg/kg feed during late pregnancy and the postpartum period until weaning, and the results showed a significant increase in daily and total milk production and the proportion of milk components of protein compared to the control group. While in other studies, zinc

supplementation [66] or spirulina algae powder [39] did not affect the quantities of milk production and milk components.

3.5. Grow Performance of Kids

The analysis of variance in Table 5 shows that the treatment of maternal goat pre-partum did not affect kids' weight at birth, but 30 days after goat kidding we found a significant increase ($P \leq 0.05$) in body weight, total weight gain and growth rate (136.90 ± 16.68%) for the offspring of goats that treated with a mixture (Zn + Sp) compared to the control group offspring. While we did not find any significant differences in the kid's growth performance of the Zn or Sp treatments compared to the control kid.

Table 5. Effect of goats' treatment with spirulina algae powder and zinc on growth performance of their suckling kids.

Parameters	Control	Zn	Sp	Zn + Sp
Weight at birth (kg)	3.18 ± 0.28 a	3.09 ± 0.14 a	3.03 ± 0.16 a	2.98 ± 0.27 a
Weight after 4 wk. (kg)	5.73 ± 0.26 b	6.46 ± 0.24 ab	6.13 ± 0.22 ab	6.92 ± 0.46 a
Total weight gain (kg)	2.54 ± 0.20 b	3.36 ± 0.21 ab	3.10 ± 0.32 ab	3.94 ± 0.35 a
Growth rate (%)	84.43 ± 13.80 b	109.73 ± 8.94 ab	105.02 ± 15.32 ab	136.90 ± 16.68 a

The values represent the mean ± standard error; Different letters within the same row are significantly differ at ($P \leq 0.05$).

The growth of suckling offspring depends mainly on the quantity and quality of the mother's milk, and accordingly, the significant increase in body weight and total weight gain may be due to the increase in the amount of milk production and milk proteins in goats that treated with Zn+Sp combined (Table 4), Which can provide the kids with the sufficient amount of nutrients and energy needed for growth. and that milk-transported Insulin-like growth factor -1 (IGF-I) can have positive metabolic effects on the liver and other peripheral tissues [67]. The improvement in the growth performance of kids may be due to the components of spirulina such as biologically active pigments (chlorophyll, phycocyanin and carotene), vitamins and minerals (selenium, zinc, copper, iron and calcium) and essential amino acids [68]. Accordingly, the treatment of lactating goats with spirulina algae improved the gene expression of protein synthesis and the cellular composition and functions of the liver in suckling kids, it also increases the total antioxidant capacity and the level of calcium in the blood and reduces the effect of toxins and heavy elements that may be transmitted from the maternal to the suckling offspring through milk [69]. Zinc is also necessary for bone growth and strength, as it stimulates gene expression of various proteins including transcription factors for differentiation into osteoblasts [70]. The mammary gland cells are responsible for providing the appropriate amount of zinc needed for the infant's growth processes during the lactation period [71]. Zinc is found in the secretory alveoli of the mammary glands, and is imported from the circulatory system, this is critical during lactation in maintaining the zinc concentration in milk, which depends mainly on maternal zinc intake [72]. The results of our study were supportive of Ragab et al. [73] who studied female rabbits and their offspring and obtained an increase in the daily growth rate of offspring after adding spirulina powder to the maternal feed at a rate of 0.6% / kg diet for 65 days. Regarding of zinc effect, Mohamed et al. [62] noted that treatment of ewes with Nano-zinc 5 and 10 mg/kg feed during late pregnancy and postpartum until weaning caused a significant increase in the body weight and average daily weight gain of lambs at birth and weaning, compared to control lambs.

Conclusion

The metabolism disturbance that results from the nutrient imbalance during the lactation period is one of the most influential conditions on the physiological state and productivity in mammals. Large and ongoing trials are still being done for the most effective control of this condition. In our current study,

we tried to investigate the effect of using spirulina algae powder and zinc alone or in combination on some physiological and productive indicators during the early lactation period of Iraqi local goats. The obtained data show that the combined use of spirulina algae powder and zinc gave positive results in some biochemical variables (Total protein, SOD and MDA), hormonal (Prolactin) and productivity (quantity and quality of milk and growth rate of offspring), which gives the possibility of using a combination of spirulina powder and zinc for ruminants in order to control some disturbed conditions with further research work using other levels or other animal species that may reveal identical or contradictory results.

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Hatch Rate, Lipid Profile, and Antioxidant Status of Broilers Fed Nano-Selenium and Vitamin E During Embryonic Stage and Exposed to a Fasting Diet

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Abstract. From January 15 to February 6, 2019, 750 fertilized eggs were used in this study, which was conducted in the hatchery owned by Al-Anwar Poultry Company in Babylon province. The eggs were divided into 5 treatments, each treatment consisting of 150 fertilized eggs; T1, as a negative control treatment without injection, and the second treatment, T2, as a positive control treatment injected with distilled water. For the T5, T4, and T3 treatments, they each received an injection of a nutritional solution containing nano-selenium and vitamin E at doses of (15-30-45 ppm, respectively). Then, the chicks were separated from the injected egg treatments, with each treatment being divided into 3 repetitions. The chicks were then grown arbitrarily for 48 hours without being fed and were only given water, and the following results were obtained: Treatment T3 significantly differentiated ($P \leq 0.01$) in percentage of hatchability, The treatment T2 ($P \leq 0.01$) was significantly excelled in the percentage of embryonic mortality compared to the rest of the experiment's treatments. There was also a significant excelled($P \leq 0.05$) for the treatment T1 in the concentration of blood cholesterol and during times 0-12-24-48 hours after hatching, a significant excelled ($P \leq 0.05$) for the treatments T2, T1 in the concentration of triglycerides during the times 0-48 hours after hatching, and the treatment T2 excelled during the times 12-24 hours, a significant excelled ($P \leq 0.05$) for the treatment T2 in the concentration of glucose Blood when hatching, and T3 and T1 treatments excelled12 hours, Treatment T2 at time 24 hours, and highly significant ($P \leq 0.01$) for T2 and T1 at 48 hours after hatching, significant difference ($P \leq 0.05$) for T5 in the concentration of GpX, was significant. Treatment T1 exceeded the concentration of Malonaldehyde at 0-48 hours after hatching.

Keywords. Early feeding, Feed fasting, Hatch rate, Antioxidant.

1. Introduction

The use of modern technology in genetics, biotechnology, and nanotechnology has significantly advanced the poultry industry over the past forty years [1] in terms of rapid bird growth and feeds

conversion efficiency, but the period of embryonic growth and development remains crucial up until hatching [2], where it was discovered that the subsequent growth and good weight during marketing are based on optimal growth during incubation and chicks' weight at hatching [3].
The survival period of the chicks in the hatchery and up until their transfer to the breeding houses, however, is a significant issue because the chicks may remain in the hatchery up until the conclusion of the counting and sorting process for a day or two without water or food, exposing them to stress and causing them to lose weight and weaken their immune systems. To increase the degree of immunity for chicks and their weight at hatching, the technology of early feeding eggs through injecting hatching eggs with vitamin, mineral, and nutrient solutions is crucial [4-7].One of the fat-soluble vitamins is vitamin E. There are eight different types of vitamin E, with a-tocopherol being the most significant due to the body's capacity to absorb and metabolize it, its antioxidant properties, which lessen the conversion of fats into peroxides and the harm caused by free radicals to cells, as well as the fact that it increases the activity and effectiveness of the enzyme glutathione peroxidase, which is responsible for lessening the formation of fatty peroxides [8].
Because to its high bioavailability and lower toxicity in comparison to its inorganic and organic forms—where inorganic compounds are more poisonous than organic compounds nano-selenium is attracting attention [9,10]. Along with protecting cells from oxidation and oxidative stress damage, nano-selenium also regulates thyroid hormone, cell growth, and antioxidant defense systems. It works in concert with alpha-tocopherol to do this. [11] spherical nano selenium has been shown to have antioxidant characteristics, which lower the risk of selenium poisoning. It can also be used as an antioxidant while lowering the risk of toxicity. [12] showed that injecting nano-selenium and vitamin E into hatching eggs enhanced the weight of the chicks and the amount of glycogen in the liver, Abdominal muscle, and heart. The aim of this study is to ascertain the impact of early nutrition with a nutrient-containing solution on the proportion of hatching, some metabolic characteristics, and nano selenium and vitamin E in broiler chick Ross 308 exposed to feed fasting in the hatchery.

2. Materials and Methods

This study was carried out in the Al-Anwar Poultry Company hatchery in the Babylon province from 15 January 2019 to 6 February 2019. 900 eggs were weighed and incubated on 15 January 2019, at the age of 17.5 days from embryo age, and 750 fertilized eggs were used after the scanning candling process for eggs was used to remove the unfertilized eggs (for each treatment 150 eggs), where T1 was used as a negative control therapy with no injections, and T2 was used as a positive control treatment with distilled water injections. They each had an injection of a nutritious solution containing nano-selenium and vitamin E at levels of (15-30-45 ppm, respectively). The chicks were grown for 48 hours after hatching, with each treatment being separated into three replicates at random and being given only water without food.

2.1. Experiment Eggs and Solutions of Injection

The eggs, with an average weight, were purchased from Al-Anwar Poultry Company's hatchery (58-60 g). Alpha-Tocopherol (Vitamin E), which is produced by the Indian HIMEDIA firm, was purchased from the office of medical and veterinary equipment in Bab al-Muadham, Baghdad province, and organic nano-selenium (liquid) was purchased from the Iranian Nanosany Corporation.

2.2. Studied Traits

2.2.1. Percentage of Hatching and Embryonic Mortality %

Calculated according to [13].

2.2.2. Biochemical Traits

The glucose was measure by use (Kit) from the German Roche company according to [14], The cholesterol concentration was measure by use (Kit) from the German Roche company according to [15], and the concentration of triglycerides was measure by use (Kit) from the German Roche

company according to [16]. As for the enzyme glutathione peroxidase, and malonaldehyde was measure by use (Kit) from the German Roche company according to [17].

2.3. Statistical Analysis

Use the statistical analysis according to the design (CRD) [18,19], and use the mathematical model:

$$Yij = \mu + Ti + eij$$

3. Results and Discussion

3.1. Percentage of Hatching % and the Percentage of Embryonic Mortality %

The percentages of hatching and embryonic mortality are affected by early feeding with nano selenium and vitamin E, as shown in Table 1. It was discovered that the T3 treatment significantly outperformed the other treatments in terms of percentage of hatching ($P \leq 0.01$) as well as the T5 treatment on the T4, T2, T1, and the two treatments T4, T1 on the T2 treatment. However, there were no significant differences between the two treatments T4 and T1. Regarding the proportion of embryonic mortality, treatment T2 outperformed T4 and T1 treatments ($P \leq 0.01$) and provided the highest average percentage of embryonic death compared to the other treatments.

Table 1. Percentage of Hatching % and percentage of embryonic mortality % in broiler chicks feed nano-selenium, vitamin E during embryonic stage.

Treatments	Means ± standard error	
	percentage of embryonic mortality %	percentage of Hatching %
T1	12.42±0.42 b	87.58±0.42 c
T2	16.40±0.93 a	83.59±0.93 d
T3	4.16±0.83 d	95.83±0.83 a
T4	12.39±0.73 b	87.60±0.72 c
T5	9.17±0.83 c	90.83±0.83 b
Significant level	**	**

The different letters differ significantly at the level ($P \leq 0.01$). The treatments (T1, T2, T3, T4, T5) was a negative control treatment without injection a positive control treatment with distilled water injection, , an injection of a nutrient solution including vitamin E, and nano-selenium at concentrations of (15, 30, and 45 ppm).

3.2. Cholesterol Concentration (mg / 100ml blood)

The blood cholesterol concentration of broiler chicks exposed to food fasting is affected by hatching eggs injected with nano selenium and vitamin E, as shown in Table 2. A significant difference ($P \leq 0.05$) was seen between treatment T1 and treatments T3, T4, and T5 at the time of hatching, but not between treatment T2 and treatment T1 or between treatments T2, T3, T4, or T5. It considerably outperformed at 12 hours after hatching ($P \leq 0.05$) T1 treatment outperformed T3 treatment as well as T5 treatment, whereas T4 on T3 treatments, and T2 treatment outperformed T3 treatment as well as T5. Also, there was no discernible difference between the T5 and T4 therapies or the T2 and T1 treatments.

Table 2. Cholesterol level (mg / 100ml blood) in broiler chicks feed nano-selenium, vitamin E during embryonic stage.

Treatments	Means ± standard error (g)			
	at hatching	12 hours	24 hours	48 hours
T1	2.44± 1.33 a	238.76± 2.39 a	244.16± 1.69 a	251.84± 1.35 a
T2	222.51± 2.36 ab	234.79± 3.62 ab	213.61± 1.36 ab	212.07± 2.94 ab
T3	198.33± 1.18 b	205.14± 5.01 c	204.41± 2.26 ab	195.28± 0.61 b
T4	187.15± 3.02 b	213.09± 1.90 b	209.45± 1.20 ab	216.97± 1.72 ab
T5	199.23± 1.88 b	216.60± 2.51 b	175.22± 1.04 b	188.39± 3.17 b
Significant	*	*	*	*

The different letters differ significantly at the level (P ≤0.05). The treatments (T1, T2, T3, T4, T5) was a negative control treatment without injection a positive control treatment with distilled water injection, , an injection of a nutrient solution including vitamin E, and nano-selenium at concentrations of (15, 30, and 45 ppm).

3.3. Concentration of Triglycerides (mg / 100ml blood)

Table (3) shows the impact on the triglyceride concentration in broiler chicks exposed to meal fasting of injecting nano selenium and vitamin E into viable eggs. When compared to the T2, T1, T5, T4, and T3 treatments, significant development was seen (P ≤0.05) for the T2 and T1 treatments at hatching. T5, T4, T3, and T2 have a substantial difference from each other. During the period of time 12 hours after hatching, it was discovered that there were substantial differences (P ≤0.05) between treatment T2 and the other transactions, as well as treatment T1's superiority over the two T5 and T4 treatments. There was no discernible change. Regarding the 24-hour period following hatching, between T3 and T1 treatments and between T5 and T4 treatments.

Table 3. Triglycerides level (mg / 100ml blood) in broiler chicks feed nano-selenium, vitamin E during embryonic stage.

Treatments	Means ± standard error			
	At hatching	12 hours	24 hours	48 hours
T1	162.24± 1.91 a	123.64± 1.48 b	106.93± 3.59 b	117.27± 3.89 a
T2	176.88± 1.27 a	136.87± 2.31 a	115.27± 3.42 a	113.63± 1.48 a
T3	100.17± 5.00 b	103.27± 5.02 bc	77.63± 2.53 c	80.37± 1.79 b
T4	92.87± 2.28 c	110.61± 4.58 c	96.01± 0.99 bc	68.66± 1.53 c
T5	92.08± 5.10 c	91.00± 1.00 c	78.15± 3.50 c	78.82± 1.80 c
Significant	*	*	*	*

The different letters differ significantly at the level (P ≤0.05). The treatments (T1, T2, T3, T4, T5) was a negative control treatment without injection a positive control treatment with distilled water injection, , an injection of a nutrient solution including vitamin E, and nano-selenium at concentrations of (15, 30, and 45 ppm).

3.4. Glucose Sugar Concentration (mg / 100ml blood)

The impact of early feeding broiler chicks with nano selenium and vitamin E on their blood sugar levels while they are fasting from food is seen in Table (4). After the eggs hatched, treatment T2 showed a significant improvement (P ≤0.05) compared to treatment T3, but there was no evidence of a significant difference between treatments T1, T2, T3, or T4. The T3 and T1 treatments considerably outperformed the T5 and T4 treatments at 12 hours after hatching (T 0.05). T2 treatment was superior to T5, T4 treatment, and the table showed no significant changes in the 24-hour period following hatching (T 0.05). No difference also occurred between T3, T2, and T1 treatments.

Table 4. Glucose level (mg / 100ml blood) in broiler chicks feed nano-selenium, vitamin E during embryonic stage.

Treatments	Means ± standard error			
	At hatching	12 hours	24 hours	48 hours
T1	156.12± 0.04 ab	188.43± 2.50 a	150.25± 1.18 ab	203.14± 4.86 a
T2	190.80± 2.56 a	160.77± 1.22 ab	184.93± 1.21 a	207.37± 3.14 a
T3	124.70± 2.45 b	149.17± 1.01 a	152.50± 2.25 ab	150.37± 0.91 b
T4	156.54± 1.57 ab	156.30± 2.12 b	134.56± 4.56 b	135.64± 4.52 b
T5	152.17± 2.98 ab	135.68± 2.49 b	139.07± 3.12 b	139.77± 1.38 b
Significant	*	*	*	**

The different letters differ significantly at the level (P ≤0.05). The treatments (T1, T2, T3, T4, T5) was a negative control treatment without injection a positive control treatment with distilled water injection, , an injection of a nutrient solution including vitamin E, and nano-selenium at concentrations of (15, 30, and 45 ppm).

3.5. Glutathione Peroxidase and Manuldehyde Concentration (IU / L)

The effect of early feeding broiler chicks with nano selenium and vitamin E on their concentrations of glutathione peroxidase and malonaldehyde is shown in Table (5). When compared to other treatments, treatment T5 significantly outperformed treatments T4 T3, T2, and T1, but there was no discernible difference between the T3, T2, and T1 treatments, as shown in the enzyme glutathione peroxidase at hatching (P ≤0.05) When compared to the other trial treatments, the T5 treatment excelled (P ≤0.05) while the T4 and T3 treatments outperformed the T2, T1 treatments. There was no discernible difference between the two treatments T4 and T3, and the treatment T2 outperformed the treatment T1.

Table 6. Glutathione peroxidase and malonaldehyde (IU / L) in broiler chicks feed nano-selenium, vitamin E during embryonic stage.

Treatments	Means ± standard error			
	Glutathione peroxidase		malonaldehyde	
	At hatching	48 hours	At hatching	48 hours
T1	64.28± 2.50 c	48.94± 1.34 d	14.96± 0.56 a	11.33± 0.84 a
T2	64.61± 1.76 c	56.93± 1.27 c	11.78± 2.50 ab	9.06± 2.63 b
T3	66.82± 2.58 c	65.96± 2.27 b	8.85± 4.10 b	6.63± 1.51 c
T4	70.65± 2.81 b	64.64± 4.40 b	9.86± 2.29 b	6.49± 2.17 c
T5	78.63± 1.21 a	80.83± 1.99 a	4.16± 0.31 c	5.23± 0.32 c
Significant	*	*	*	*

The different letters differ significantly at the level (P ≤0.05). The treatments (T1, T2, T3, T4, T5) was a negative control treatment without injection a positive control treatment with distilled water injection, , an injection of a nutrient solution including vitamin E, and nano-selenium at concentrations of (15, 30, and 45 ppm).

4. Discussion

The antioxidant activity of both Vitamin E and nano selenium may be the source of the improvement in hatchability in therapies involving injections of these substances [20] showed that nano selenium enhances antioxidant status by triggering the enzyme glutathione peroxidase, [21] said to have higher antioxidant activity are spherical nano-selenium nanoparticles with sizes ranging from 100 to 500 nm [22] where they excel in neutralizing free radicals [23], maybe the fact that vitamin E prevents cells from oxidative stress may be the reason why the experiment chicks were able to hatch [24]. Where [25] indicated that the injection of hatching eggs with nano selenium at a concentration of 15 ppm improved the weight of the incubated chicks and increased the average hatch rate in relation to reducing lipid oxidation within the body and the production of free radicals, A decrease in the average level of thyroid hormone Tri-Iodine T3 Thyroxine T4 secretion may be the cause of the rise in

cholesterol and triglyceride levels in the blood of treatment T1 birds. This rise may also be caused by the exposure of birds to oxidative stress brought on by fasting and an increase in lipid peroxide, Nano selenium and vitamin E have a direct effect on increasing the production and metabolism of thyroid hormones, which may be a reason for the decreased cholesterol in the blood plasma of nano selenium and vitamin E-injected chicks. As the reducing activity of thyroid glands generally leads to an increase in the level of cholesterol in the blood by decreasing both the average of cholesterol formation and the average of its excretion in the bile, [26,27].

As for the reduction glucose level in T5, T4 treatments, it may be due to the role of the antioxidant of nano selenium and its effect on thyroid hormones and its effect on body metabolism and glucose [26,27,28] or it may be caused by ability Vitamin E enhances the role of antioxidants in the cell and reduces the effect of oxidative stress, which activates the work of body cells, including pancreatic beta cells, and then activates insulin secretion that lowers blood glucose levels [29], As birds need high blood glucose during stress for the purpose of resisting it, and that blood glucose level decreased significantly in birds injected with vitamin compared to control and positive control birds, this may be due to the anti-stress effect resulting from the fasting of vitamin E, which led to a decrease in the ACTH level hormone In the blood of birds treated with vitamin and to decrease the effectiveness of protein destruction for the purpose of developing sugar, which leads to a decrease in blood glucose [27]. Vitamin E also works as a defensive line to preserve the body from the harmful effects of free radicals, thereby protecting cell membranes, especially its acids. Unsaturated fatty from the harmful effects of oxidation such as cell damage or turn it into abnormal cells [30].

[31] have observed a positive linear relationship between the concentration of vitamin E in the body and glucose metabolism and suggested that this may be due to the role of the vitamin in stimulating glutathione enzyme and increasing the concentration of Mg within cells and then regulating cellular metabolism especially for the glucose metabolism that plays magnesium There is a major role in activating the hormone insulin, Regarding the rise in the concentration of glucose in the treatment T1, this rise in the concentration of glucose may be brought on by the birds' exposure to food stress and the production of glucose sugar from non-carbohydrate sources, as well as by the rise in the decomposition of glycogen brought on by the rise in the secretion of hormones that stimulate the enzymes that break down glycogen (epinephrine, norepinephrine, and glucagon). These hormones are generated in reaction to stress in order to meet the body's energy requirements both under stressful or fasting conditions and to maintain a reasonably high level of blood glucose, which is the primary fuel for the brain and nervous system. It is clear that the nutritional solution containing nano selenium and vitamin E, which was injected into the treatments, reduced malonaldehyde and increased glutathione peroxidase when compared to the positive and negative control treatments. This suggests that the antioxidant nano selenium may play a role in these results [32-34].

[35] noticed that a lack of nano selenium results in an increase in the production of free radicals and a loss in the body's ability to protect itself from oxidative processes; oxidative stress is a common term for this state; due to the dependence of nano selenium on glutathione peroxidase (GPx) most important for detoxification of hydroperoxides, and its activities have been widely used as bio parameters to assess selenium status in birds, it has been shown that determining the activity of glutathione peroxidase is an effective way to estimate the biological availability of nano selenium that glutathione peroxidase depends Its composition is based on nano selenium, which in turn protects neutral cells and other blood components against peroxide oxidation. The reason for the improvement in the injections treatments Using vitamin E and nano selenium, the proportion of eggs hatching and Lipid profile and antioxidants compared to the control treatments, is because the injection of the nutrient solution into the Amniotic fluid and its ingestion by the embryo in the last third of the incubation (19 days), causing the Digestive Tract activity [12] increased tissue protection against oxidation of fats and proteins [36] as antioxidants, which in turn decreased the severity of oxidative stress. The effect was evident after hatching chicks, where the results of lipid oxidation processes in nano-selenium and vitamin E injections decreased due to their antioxidant role. Including vitamin E and nano selenium creates an integrated antioxidant system that acts to defend embryonic cells from the damaging effects of free radicals [37] Although vitamin E and nano selenium do not include oxidative processes or their impact on metabolism [38]. The chain of peroxides produced by lipid oxidation processes, which results in

free radicals damaging to the embryo, is broken down by vitamin E [39,40], and it is reported [41] a representation of 80% vitamin E in embryonic tissue Tocopherols are the most active form in embryonic tissue.

Conclusions

The findings demonstrate that vitamin E and nano selenium increased the percentage of hatching, decreased the oxidation state, and enhanced the body's lipid profile. Further research is required to determine how nano selenium affects the physiological characteristics and growth performance of chickens.

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Estimation of Genetic Parameters for Monthly Egg Production and Egg Weight in Iraqi Indigenous Brown Chickens

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Abstract. A study was conducted to estimate the genetic parameters of monthly egg production and egg weight of Iraqi indigenous brown chickens. Data were descended from 2234 females of six-generation selected for high egg production. Co-variance components were estimated based on the Average Information Restricted Maximum Likelihood [AI-REML] algorithm of Wombat software. Egg numbers were measured every four-week intervals while, egg weight was recorded for three periods, average first three months, second three months, and a total of six months. Egg number was 10.07, 20.46, 21.17, 20.12, 19.37, 18.13, and 109.32 egg\hen from moth 1 to 6 and cumulative production respectively. Heritability and standard errors of monthly egg number were 0.39±0.05, 0.29±0.04, 0.18±0.04, 0.36±0.06, 0.22±0.05 and 0.17±0.04 for first, second, third, fourth, fifth and six months respectively while it was 0.40±0.05 for the cumulative period. Average egg weights for the first 12 weeks, second 12 weeks, and cumulative partial record period [24 weeks] of production showed higher heritability estimations [0.60±0.05, 0.71±0.04, and 0.68±0.04 respectively]. Genetic and phenotypic correlations between monthly egg numbers ranging from low to high positive estimations. Genetic correlations between monthly and cumulative part record egg numbers were positive and high ranging from 0.57±0.07 to 0.89±0.04. Genetic correlations between egg weights at different periods were much higher and tend to be close to the unity. Genetic correlations among monthly egg production and egg weight for three periods were negative and low in general ranging from -0.02±0.09 to -0.48±0.14. Based on the current estimation for genetic parameters and lower standard errors indicate that the estimates have higher precision and may applying in a breeding program for improvement of Iraqi indigenous brown chickens' performance.

Keywords. Egg weight, Heritability, Genetic and phenotypic correlations, Monthly egg production, Local chickens.

1. Introduction

Egg production is a crucial single trait in layer breeding because it determines the egg output in a certain period of the hen's lifetime [1]. However, no other trait has the importance of egg production rate as a sign of egg layer efficiency. On the other hand, with no production, the others traits cannot be measured. Therefore, great emphasis must be paid to select for increasing the rate of egg production in local

chickens. Local chickens in many developing countries produce a lower rate of egg production with many pauses in the clutch length due to broodiness or unfavorable environmental conditions. Clutch length is the number of eggs laid on consecutive days. Compared with the commercial standard layer, the shape of the production curve in Iraqi brown local chickens has not followed the standard three essential stages of production over time. In this regard, some hens that reached sexual maturity at an early age may achieve steady production in one or two months and then decline with little or no egg production which caused higher variation among birds in all production stages [Personal observations]. Selection for increasing egg production within local chickens may be a pivot tool to enhance egg production [2-4]. Many genes influenced and controlled egg production shape during the lifetime of the laying cycle. The gene expression varied with age [5,6] which caused the variation in egg production at the beginning and the end of the production cycle. Therefor the values of heritability and correlations for egg production may not necessarily be the same in the initial and the end of the production cycle [6]. [7,8], estimates higher heritabilities in the first month of production cycle but were lower in the other successive months. Dana et al [9] found differential estimates of heritability for monthly egg production in local Horro Ethiopian chickens. In this regard, Anang et al. [7] advised to use monthly egg production over the cumulative production in genetic evaluation of layer chickens. In the same way, Wolc et al [6] concluded that the estimation of genetic parameters based on cumulative egg production was not sufficient enough to explain this trait. Heritability is a ratio of genetic variance to phenotypic variance and measure the relation between individual phenotype and its genetic makeup. The present work was initiated in 2014 to improve the egg output of Iraqi brown local chickens through the selection of birds with high egg numbers. Iraqi local chickens are valuable genetic resources and showed good adaptable to the Iraqi harsh climate and have favorable features [10-15]. Estimation of genetic parameters are effective tool to aid breeding plan. In selected populations, the mixed model methodology under REML has theoretical advantages for the estimation of genetic parameters [16]. On the other hand, Selection based on part records [from onset of lay to 40 weeks of age] to improve annual egg production was achieved [17] because higher and positive genetic and phenotypic correlation between part and annual egg production. Therefore, the current work was conducted on the Iraqi local chicken populations under selection for egg production based on partial record to estimate genetic parameters of the egg production and egg weight via an animal model [18] with a comprehensive study to enrich the scarce information on the local chicken genetic parameters.

2. Material and Methods

2.1. Study Site

This was carried out in Poultry Research Station at the Office of Agricultural Research /Ministry of Agriculture was used. The poultry farm is located at Longitude 33˚, 312,313'E and Latitude 44˚, 202,868'N. The birds of the current population were sires and dams of six generation select individually for high egg production.

2.2. Chicken Population

The first generation was obtained from the base population reared randomly at the Poultry Research Station\ Office of Agricultural Research \ Ministry of Agriculture. Five families of 10 females and one male were established to compose the first generation. The hatching eggs were collected two weeks after mixing the males with the females and incubated separately for each family. After 21 days of incubation, offspring were obtained and raised in a separate pen to each family until the age of 17 weeks. At 17 weeks of age, they were transferred to the individual cages [40 x 60 x 40 cm] in order to identify the productive performance of each hen. From this generation onwards, artificial insemination was carried out to get hatching eggs. All hatching eggs were numbered and recorded with sire and dam number. All chicks hatched was wing-banded and reared in group based on their sir’s and dam’s.

2.3. Environment and Feeding

Birds of this study were reared in floor semi-closed house equipped with brooding heaters, feeders, waters, and a lighting system. The wood shaving was bedded on the house floor. The temperature and relative humidity were controlled as much as possible to achieve a proper environmental condition for each age in the house. Feed and water were offered freely. Five diets were introduced to chicks from hatch to the production phase. The starter diet [20% CP and 2900 Kcal/kg feed ME] from hatch to 4 weeks, the grower diet [17% CP and 2750 Kcal/kg feed ME] from 4 to 10 weeks, the developer diet [16% CP and 2750 Kcal/kg feed ME] from 10 to 16 weeks, pre-layer [16% CP and 2750 Kcal/kg feed ME] from 17 to 5% egg production and layer diets [17% CP and 2800 Kcal/kg feed ME] from 5% to the end of experiment were fed on mash or crumble form. The compositions of the diets have not appeared in the separate table because changes in ingredients have happened across generations. All birds provided with light regimen with dark and light program according to their age. Birds were vaccinated against Marek disease, ND, IBD, fowl pox, and AE.

2.4. Data

Data used in this study represent six years of hatch [from 2016 to 2022]. Parents of offspring were known for each bird, and six generations of pedigree were available for all birds with records. The overall number of animals in the pedigree file was 2480. The number of animals after pruning was 2450. The 216 animals without records were excluded from analysis Therefore, pedigree records of the remaining 2234 hens were tested (Table 1). Egg production [EP] and egg weights [EW] were recorded individually on a daily basis and data were summarized on monthly interval for EP and every 12 weeks' interval for EW. Hens without record for entire production cycle were excluded from analysis.

Table 1. Pedigree information of data used in the genetic parameters estimation.

Pedigree information file	n
Number of animals in pedigree file	2480
Number of animals after pruning	2450
Proportion % remaining	98.8
Number of levels without records	216
Number of levels with records	2234
Number of animals without offspring	1449
Number of animals with offspring	1000
Number of animals with record	785
Number of sires	152
Number of dams	849
Generations	6
Hatch	2
Season	4

2.5. Statistical Analysis

Descriptive statistics on studied traits were analyzed by using SAS software [19]. Three-way analysis of variance without interaction through the generalized linear modeling [GLM] procedure of SAS was used to analyze the quantitative data. Generation, hatch, and season showed highly significant on studied traits and were included in the model as fixed effect. Significant differences were considered at level of $P<0.05$. Means were separated by using Duncan's multiple range and multiple F tests.

Genetic parameters were estimated based on univariate and bivariate animal model. The single animal model of Wombat package [18] was used to estimate variance components with the following model:

$$Y = Xb+Za+e$$

Where: Y = observation's vector of the trait, b = vector of fixed effects [generation, hatch and season], a, is the vectors of direct additive genetic effect and e = vector of random residual effect, X and, Z are incidence matrices relating records to the fixed and direct additive genetic effect.

The variance components for the random effects were denoted as var [a] = $A\sigma^2a$ and var [e] = $I\sigma^2e$

where A is a numerator relationship matrix. Bivariate animal model was used to estimates genetic and phenotypic correlation between studied traits with the matrix notation of:

$$\begin{pmatrix} y1 \\ y2 \end{pmatrix} = \begin{pmatrix} x1 & 0 \\ 0 & x2 \end{pmatrix} \begin{pmatrix} b1 \\ b2 \end{pmatrix} + \begin{pmatrix} z1 & 0 \\ 0 & z2 \end{pmatrix} \begin{pmatrix} a1 \\ a2 \end{pmatrix} + \begin{pmatrix} e1 \\ e2 \end{pmatrix}$$

Where: y1 and y2 is a vector of observations, b1 and b2 is a fixed effect on traits, a1 and a2 is a random additive genetic effects on traits, e1 and e2 is a random residual error. X and Z is the incidence matrices related to fixed and random effect respectively.

3. Results

3.1. Descriptive Statistics

Egg number and egg weight and their standard deviation, coefficient of variation, and minimum and maximum value were presented in table 2. Egg number was 10.07, 20.46, 21.17, 20.12, 19.37, 18.13, and 109.32 egg\hen from moth 1 to 6 and cumulative production respectively. The standard deviation for monthly EN ranged from 4.79 to 8.15, whereas, it was 21.71 for the overall six months. A higher coefficient of variability [CV%] was shown in the first month which revealed the variations between hens in reaching sexual maturity. Average egg weights were 42.07, 47.28, and 44.80g for the first three months, second three months, and overall six months respectively. The standard deviation and CV% for EW were in acceptance levels which is an indicator of the uniformity of this trait.

3.2. Non-Genetic Effects

The effect of generation, hatch, and season on monthly, cumulative egg production and average egg weight was highly significant [Table 3]. Monthly egg production varied between generations. In the first month of production, the highest egg number [EN] was recorded in the fourth generation [15.09 egg/hen] while the lowest was in the second generation [3.45 egg/hen]. However, in the six months of production, the highest value was recorded in the fifth generation [19.55 egg/hen] while the lowest was in the first generation [15.34 egg/hen]. The cumulative EN from the onset of lay to 40 weeks of age varied between generations where the highest EN was recorded in the six-generation compared to the first generation [112.45 vs 104.87 egg/hen]. EW achieved better values in the second generation[47.60g] while the lowest was recorded in the fourth to the six generations [ca 43.0g]. EW was increased with age progressive. The hatch effect had a significant effect on EN and EW. The EN per hen across months, in general, tends to be highest in the first hatch compared to the second hatch. The cumulative EN in the first hatch was greater compared to the second hatch [111.94 eggs vs 106.36 eggs]. Furthermore, the average EWT in the first hatch was greater than those in the second hatch. The effect of season was significant on EN and EW. The overall EN for the hens hatched in the spring season achieved significantly lower egg production than their counterparts hatched in the other season times. Whereas, the scenario was the opposite for the overall average EW where the highest values were recorded in the spring season and the lowest in other seasons.

Table 2. Descriptive statistics for egg production [number/hen] and egg weights [g] of Iraqi Indigenous chickens.

TRAIT	Records, n	Mean	SD	Minimum	Maximum	CV%
EPM1	2234	10.07	8.15	0	33[1]	81.01
EPM2	2234	20.46	5.88	0	28	28.73
EPM3	2234	21.17	4.79	0	28	22.61
EPM4	2234	20.12	4.94	0	28	24.55
EPM5	2234	19.37	5.08	0	28	26.25
EPM6	2234	18.13	5.64	0	27	31.11
EPM1-M6	2234	109.32	21.71	3	156.00	19.86
EWTM1-M3	2234	42.07	3.22	24.00	54.21	7.65
EWTM3-M6	2224	47.28	3.43	32.69	61.05	7.25
EWTM1-M6	2234	44.80	3.14	30.31	59.05	7.40

1: this value was higher due to include the age of sexual maturity.

Table 3. Effect of generation, hatch and season on egg production [egg/hen] and average egg weight [g] of Iraqi brown local chickens [mean± SEM].

Factor	Record	Trait[1]									
		EPM1	EPM2	EPM3	EPM4	EPM5	EPM6	EPM1-6	EWM1-3	EWTM3-6	EWTM1-6
Generation											
1	289	10.27±0.45 c	21.79±0.30 a	20.28±0.36 c	18.99±0.39 c	18.20±0.39 c	15.34±0.44 e	104.87±1.62 b	42.94±0.18 b	47.71±0.18 c	45.23±0.18 c
2	220	3.45±0.37 e	15.92±0.54 d	21.30±0.28 ab	21.41±0.41 a	20.78±0.26 a	19.04±0.34 ab	101.98±1.32 b	44.84±0.20 a	49.42±0.24 a	47.60±0.22 a
3	401	8.34±0.36 d	20.73±0.24 bc	21.88±0.16 a	21.37±0.15 a	20.77±0.18 a	18.08±0.26 cd	111.17±0.83 a	43.09±0.15 b	48.68±0.16 b	46.10±0.15 b
4	433	15.09±0.39 a	21.33±0.26 ab	20.32±0.27 c	18.85±0.27 c	18.21±0.28 c	17.37±0.28 d	111.16±1.19 a	41.32±0.14 c	46.49±0.16 d	43.85±0.15 d
5	577	8.57±0.32 d	19.99±0.25 c	21.79±0.16 a	20.48±0.18 b	19.60±0.19 b	19.55±0.18 a	109.98±0.75 a	40.09±0.11 d	46.71±0.12 d	43.95±0.11 d
6	314	12.54±0.43 b	21.73±0.31 a	20.99±0.31 bc	19.77±0.28 b	18.83±0.30 c	18.59±0.30 bc	112.45±1.21 a	40.48±0.19 c	45.77±0.20 e	43.64±0.19 d
Hatch											
1	1185	10.92±0.24 a	20.10±0.16 a	21.10±0.14 a	20.34±0.14 a	19.74±0.14 a	18.74±0.15 a	111.94±0.63 a	41.42±0.10 a	47.59±0.10 a	45.11±0.10 a
2	1049	9.10±0.25 b	19.74±0.19 b	21.24±0.14 a	19.87±0.15 b	18.94±0.16 b	17.44±0.18 b	106.36±0.66 b	40.67±0.10 b	46.92±0.10 b	44.44±0.10 b
Season											
Winter	1519	10.66±0.21 ab	20.72±0.14 b	21.38±0.11 a	20.24±0.12 b	19.47±0.12 b	18.47±0.13 a	110.94±0.52 a	41.48±0.08 c	46.98±0.09 c	44.36±0.08 c
Spring	220	3.45±0.37 c	15.92±0.54 c	21.30±0.30 a	21.41±0.28 a	20.78±0.26 a	19.04±0.34 a	101.98±1.33 c	44.84±0.20 a	49.43±0.24 a	47.60±0.22 a
Summer	169	9.96±0.58 b	22.18±0.34 a	19.95±0.52 b	19.15±0.57 c	18.96±0.53 b c	18.15±0.55 a	108.35±2.24 a b	42.73±0.23 b	47.68±0.25 b	45.15±0.25 b
Autumn	326	11.80±0.41 a	21.42±0.30 ab	20.71±0.31 ab	19.22±0.29 c	18.15±0.31 c	15.93±0.37 b	107.23±1.33 b	42.59±0.18 b	47.02±0.19 c	44.76±0.18 b
Source of variation		Level of significance									
Generation		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Hatch		<.0001	<.0001	0.5784	0.0232	0.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Season		<.0001	0.0055	0.1371	0.0517	0.0452	<.0001	0.0011	<.0001	<.0001	<.0001

1: EPM1-EPM6 represent monthly egg production from one month to six months and cumulative egg production, EWTM1-EWTM3, EWTM3-EWTM6 and EWTM1-EWTM6 is an average egg weight for first three months, second three months and overall six months of production periods.

3.1. Heritabilities and Genetic and Phenotypic Correlations

Heritability of egg production and egg weight traits are shown in Table 4. Heritability estimates for monthly egg production varied from 0.17 to 0.39 and in the cumulative period was 0.40. Lower heritability values were estimated at third and sixth month and higher was at first and fourth month. The h^2 estimates for egg weight had higher values ranged from 0.60 at the average of the first three months to 0.71 at the average of the second three months. Genetic and phenotypic correlations among studied traits are presented in Table 5. Monthly egg production showed varied genetic and phenotypic correlations between each other's. The first month was moderate to high genetic correlation with the second and third month but low and negative with the fourth to sixth month. Generally, genetic correlations among egg production traits ranged from -0.04 [EPM1 with EPM5] to 1.00 [EPM5 with EPM6]. The phenotypic correlation between monthly egg production ranged from -0.003[EPM1 with EPM5] to 0.64 [EPM4 with EPM5 and EPM5 and EPM6]. The genetic [rg] and phenotypic [rp] correlation between monthly egg production and cumulative egg production were highly positive, ranged from 0.57 [EPM1 with EPM1-M6] to 0.89 [EPM3 with EPM1-M6]. While rp ranged from 0.50 [EPM1 with EPM1-M6] to 0.74 [EPM3 with EPM1-M6]. The low and moderate antagonistic relationship between egg number and egg weight traits genetically and

phenotypically was shown at all periods, ranging from -0.02 to -0.48 for genetic correlations and from -0.003 to -0.24 for phenotypic correlations. The genetic [rg] and phenotypic [rp] correlation between egg weight traits were highly positive, rg ranged from 0.94 to 0.98 and rp ranged from 0.80 to 0.94.

Table 4. Variance component and heritabilities of monthly and cumulative and average egg weights of Iraqi local chickens selected on part record [19-43 weeks of age].

TRAIT	Record, n	Animal, n	Sire, n	Dam, n	σ^2a	σ^2e	σ^2p	h^2	±se
EPM1	2234	2450	152	849	21.58	33.51	55.09	0.39	0.05
EPM2	2234	2450	152	849	9.19	22.87	32.06	0.29	0.04
EPM3	2234	2450	152	849	4.01	18.81	22.82	0.18	0.04
EPM4	2234	2450	152	849	8.82	16.03	24.85	0.36	0.06
EPM5	2234	2450	152	849	5.49	19.72	25.21	0.22	0.05
EPM6	2234	2450	152	849	5.57	26.48	32.05	0.17	0.04
EPM1-M6	2234	2450	152	849	189.7	286.39	476.09	0.40	0.05
EWM1-M3	2234	2450	152	849	5.41	3.55	8.96	0.60	0.05
EWM3-M6	2224	2439	152	846	7.84	3.18	11.02	0.71	0.04
EWM1-M6	2234	2450	152	849	6.61	3.16	9.77	0.68	0.04

[1:] EPM1-EPM6 represent monthly egg production from one month to six months and cumulative egg production, EWM1-EWM3, EWM3-EWM6 and EWM1-EWM6 is an average egg weight for first three months, second three months and overall six months of production periods.

Table 5. Genetic [above diagonal] and phenotypic [below diagonal] correlations between monthly egg production and egg weights of Iraqi brown local Chickens.

Traits1	EPM1	EPM2	EPM3	EPM4	EPM5	EPM6	EPM1-M6	EWM1-M3	EWM3-M6	EWM1-M6
EPM1		0.67[0.07]	0.25[0.12]	0.05[0.11]	-0.04[0.13]	0.03[0.13]	0.57[0.07]	-0.22[0.08]	-0.11[0.08]	-0.23[0.08]
EPM2	0.41[0.02]		0.53[0.12]	0.24[0.12]	0.34[0.14]	0.10[0.14]	0.67[0.07]	-0.10[0.11]	-0.18[0.10]	-0.19[0.09]
EPM3	0.11[0.02]	0.48[0.02]		0.95[0.04]	0.88[0.06]	0.95[0.07]	0.89[0.04]	-0.19[0.11]	-0.37 [0.12]	-0.14[0.11]
EPM4	0.05[0.02]	0.25[0.02]	0.63[0.01]		0.97[0.02]	0.96[0.03]	0.84[0.04]	-0.13[0.09]	-0.28[0.12]	-0.02[0.09]
EPM5	-0.003[0.02]	0.19[0.02]	0.47[0.02]	0.64[0.01]		1.00[0.03]	0.81[0.05]	-0.15[0.11]	-0.26[0.12]	-0.08[0.10]
EPM6	0.03[0.02]	0.09[0.02]	0.41[0.02]	0.53[0.02]	0.64[0.01]		0.84[0.05]	-0.10[0.12]	-0.48[0.14]	-0.13[0.11]
EPM1-M6	0.50[0.02]	0.65[0.01]	0.74[0.01]	0.73[0.01]	0.69[0.01]	0.68[0.01]		-0.16[0.09]	-0.27[0.09]	-0.11[0.08]
EWM1-M3	-0.23[0.02]	-0.02[0.02]	-0.04[0.02]	-0.02[0.02]	-0.01[0.02]	-0.02[0.02]	-0.08[0.03]		0.94[0.01]	0.98[0.01]
EWM3-M6	-0.10[0.03]	-0.12[0.02]	-0.12[0.02]	-0.08[0.02]	-0.06[0.02]	-0.10[0.02]	-0.16[0.03]	0.80[0.01]		0.98[0.01]
EWM1-M6	-0.24 [0.02]	-0.14[0.02]	-0.07[0.02]	-0.008[0.02]	-0.003[0.02]	-0.03[0.02]	-0.09[0.03]	0.93[0.003]	0.94[0.003]	

1: EPM1-EPM6 represent monthly egg production from one month to six months and cumulative egg production, EWM1-EWM3, EWM3-EWM6 and EWM1-EWM6 is an average egg weight for first three months, second three months and overall six months of production periods.

4. Discussion

The phenotypic traits related to egg number and egg weights of Iraqi local chickens are somewhat greater than local chickens in other worldwide area. In the current study, egg production increased steadily from the second month to the fifth month. The trend of egg production observed in the present study revealed a positive response to selection on a part-record basis. Selection for early period part-records [from the onset of lay to 40 weeks of age] is a crucial approach for improving egg production in egg-type chicken flocks to make a substantial genetic improvement [20]. The range of egg production in some local chickens in Asia [21, 22, 23, 24, 25] or in Africa [26, 2, 9] showed lower part-record egg production than in the current population. In Iranian [27] and Thai [4, 28] indigenous chickens, the egg number laid per hen from the onset of lay to 12 or 17 weeks of the production period was 35.10 or 55 to 59.31 eggs respectively. The production performance of the current population is close to the performance of different lines of white leghorn selected for egg numbers [29].

The mean of egg weight in the current study was in agreement with previous studies [2, 22, 30, 31] that showed the range of weights was in the forties. The egg weight increased with age advancement. Niknafas et al. [3] also showed this trend in Mazandaran native chickens. In global indigenous chickens [32, 33, 34], the EN and EW was differing significantly due to the generation effect. They showed that egg production and egg weight increased over a generation. In the current study, egg production was increased and egg weight was decreased across generations due to the selection criterion being dependent mainly on the direct selection of birds with high EP rather than EW. The significant increase in EN and decrease in EW over generations is a result of the antagonist relationship between egg production and egg weight.

The hatch effect on EN and EW was significant over the generations, where the first hatch performs better than the second hatch, which might be due to the different environmental factors related to the house effect. The second hatch is routinely raised in the same house as the first hatch and many environmental conditions such as temperature, humidity, and lighting regime could affect negatively performance [33]. In the present work, the effect of the season on EN and EW was significant. In the present work, hens hatched in the spring season performed lower than their counterparts hatched in other seasons. The reason might be differences in climatic conditions exposed to the bird. birds that hatched in the spring season grow in a moderate climate and are introduced to the lay in the harsh summer season which caused a negative on performance even with using good sources of house cooling. Previous research [35] showed that hens kept in cages during the summer and autumn seasons produced more eggs compared with those in the spring and winter seasons, but in the aviary system [organic system] the higher production was in the spring season. Also, Mugnai et al. [36] found that hens kept in cages during the spring season produced more egg mass per day compared to those in summer and autumn.

In the current research, estimated heritabilities of monthly egg number ranged from 0.17 to 0.39, and for overall periods was 0.40. High heritability in the first month is a result of the effect of sexual maturity variations as shown previously [8, 9],

The cumulative heritability for egg production in this work was greater than reported for Thai indigenous chickens [4,37] and Iranian Mazandaran native chicken [3]. However, the results are comparable to Korean native chickens [21], and Ethiopian indigenous chickens [9]. Meanwhile, in commercial egg-laying chickens [6] heritability ranged from 0.02-0.03 in the repeated model and 0.1 in the cumulative model. The higher heritability estimated for cumulative EN in the current study is in part as same as reported in Iranian Fars native chickens [31], White leghorn chickens [7, 8]. Moderate heritability for egg production and higher heritability for egg weight indicate that selection for higher egg number and/or egg weight could be applied to improve the performances of Iraqi local chicken. Changes in heritability over time may result from the activation of different genes during the production cycle as reported by Anang et al. [38], Wolc and Szwaczkowski [39], and Dana et al. [9]. Heritability values for EW in the present results are close to the value reported by Kamali et al. [31] and higher than those reported by Nurgiartiningsih et al. [8] and Niknafis et al. [3]. Higher heritability for EW could be a good indicator to select birds directly in order to improve this trait.

In the present study, monthly egg production showed varied genetic and phenotypic correlations. The first month was correlated genetically and phenotypically in varied magnitudes where high or moderate with the second and third months but low or negative with other months. Whereas, in the rest month the

high and positive genetic and phenotypic correlations were recorded. This trend was also investigated by Nurgiartiningsih et al. [8, 40], Wolc et al. [6], and Dana et al. [9]. Furthermore, the positive and high genetic and phenotypic correlations between monthly EN and cumulative EN were reported in the previous reports [8, 21, 2, 9]. Egg weight traits appeared to be correlated negatively with EN and positively with each other. This magnitude is consistent with that presented in the literature [8, 21, 22, 31]. Review of literature shows that additive genetic variation increased with age for egg number egg weight, and albumen height [41]. Also, Ledur et al. [5] stated that genetic variance of egg production increased with age.

Conclusion

The good performance related to egg production and egg weight in Iraqi brown local chickens that showed in the current work is essential for preserving and conserving these gene pools as a reserve on the one hand and important for local producers to enhance egg production operations on the other hand. Furthermore, the higher genetic parameter estimates based on partial records may be the most efficient strategy for maximizing egg production in laying hens compared to annual records. The higher heritability and positive genetic correlation between egg weights suggest that this trait could be improved through the selection. The antagonist relationship between EN and EW traits is an indicator of improving each trait in a different manner. The standard errors of estimates in this study were low indicating that the estimates may have sufficient precision and reliability.

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Detection of Deoxynivaenol (DON) Toxin in some Samples of Corn Chips and Corn Flex Collected from Local Markets

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Abstract. Eighteen samples of yellow corn chips, popcorn and corn flex of various origins were collected from local markets in Tikrit. In order to detect the fungal contamination of these samples, it cultured on the potato dextros agar (PDA), with three replicates for each sample. Also, the test for the presence of Deoxynivalenol toxin (DON) were done, using an ELISA kit. The results were showan that there are five types of fungi that contaminate the samples under study with varying proportions. It was found that all samples were contaminated with *Penicillium spp*., while Nine samples were contaminated with *Aspergillus niger*,and. Six samples were contaminated with *Fusarium spp*.. However, *Cladosporium herbarum* was appeared in three samples only, and *Aspergillus tiresias* was present in only two samples. The results for the detection of DON toxin were shown that all samples were contaminated with DON toxin but with dissimilar concentrations. The highest concentration (3.69 mg/kg)was found in the Buwak Gypsum sample, but the lowest concentration (71.5 mg/kg) was found in the Egyptian corn flakes sample. All concentrations of DON toxin found in the samples were higher than the allowable average value (0.3-2.0) mg/kg) in wheat and maize grains, and (0.75 mg/kg) in wheat flour, these values were approved by the European Union countries.

Keywords. Deoxynivaleon toxin, *Fusarium graminearum*, Corn flex, Chips.

1. Introduction

Mycotoxins are toxic as a secondary metabolites produced by many types of fungi which contaminate field crops [1]. Fungal development and mycotoxin production occurs under suitable humidity and temperature conditions during field or storage periods [2]. Mycotoxin contamination occurs extensively on the foods of plant origin, especially grains, fruits and feeds for animal consumption.The most important of these toxins are aflatoxin, fumonisin, zearalenone and deoxynivalenol(DON) [3,4]. These toxins are produced by species of fungi belonging to the genus. *Aspergillus, Penicillium, Fusarium* [5].

DON is a natural mycotoxin produced primarily by *Fusarium graminearum* and also is known as vomitoxin due to its strong emetic effect after consumption [6]. This toxin is one of the most common trichothecene mycotoxins, and is a major contaminant of grass grains and animal feed [7]. The risk of the exposure to DON toxins comes from eating food contaminated with the toxin directly, such as contaminated grains and cereal products, or indirectly from consuming milk, eggs, or meat from

animals that have been fed a toxin-contaminated feeds. The emportant factor,that these toxins, have the property of accumulating overtime within the tissues of living organisms [8].

DON has been found in varying proportions in maize and corn products such as popcorn and potato chips and other food products such as flour, bread and baby food.[9]. DON poisoning causes nausea, fever, vomiting, headache, abdominal pain, and dizziness, and thus affects the metabolic processes and energy production in the human body [9]. Wheat, corn and other grains had acceptable levels (0.3-2 mg/kg) of DON, while it was 0.75 mg/kg in ready-to-eat flour. These levels have been approved in European Union countries [10]. Wood (1992) confirmed that DON toxin contaminates 85% of cereals and foods at a rate of 2 μg/g [11].

In a study [12] that included 26 samples of maize products available in local markets in Indonesia to detect mycotoxin contamination, they found that all samples included in the study were contaminated with mycotoxins, especially Deoxynivalenol.

In a study [9] that included maize products of different origins to estimate their contamination with the fungus and DON toxin, the study showed that all samples were infected with fungi of the genus *Fusarium* and *Trichodema spp*. and *Rhizopus* and *Stachybotrys spp*. The author found that all samples were contaminated with DON. The researchers pointed out that it is almost impossible for corn or its products to be free from mycotoxins or to prevent contamination with them [13].

Due to the weak role of food control and the absence of the quality control over most food sources and most crops storage methods, especially foodstuffs ingredints that children usually consumption a lot, such as corn chips and corn flakes, as well as the openness Iraqi markets of food products without central quality control. More over, there is an obvious complacency from the local governerate for consumer protection from unsafe food products. According to the above failure excuses, the aim of this study is to highlight on the contamination of foods(corn chips,cornflex,and popcorn) that consumed daily by children and school students with widespread maner, with fungi and toxins secreted by these fungi.Taking into account, that these toxins are believed to be cumulative. In order to implement the above goal ,the following practical steps have to be followed :

Collecting a number of samples of corn chips, corn flakes and pop corn obtained from local market,cultavation and isolating the fungi that contaminated the collected samples on a suitable and selective media and quantative assessment of DON toxin in collected samples by ELIZA technique.

2. Materials and Methods

2.1. Sample Collection

Eighteen samples of corn chips, popcorn, and corn flex were collected from the local markets of Tikrit city, with three replications for each sample.

2.2. Sample Preparation

Three grams were taken from each sample with three replicates for each sample, then, it was grinded well with a sterile mortar. An amount of 1 grind gm was taken from each sample and placed in a sterile test tube with 10 ml of physiological solution was added to get the first dillution of the sample. Then a serial dilution process was performed up to third dilution. Then 1 ml of the last two dilutions were taken and did culturing process on Potato Dextrose Agare (PDA) medium, using the surface inoculation method according to the method mentioned by [24,25].

2.3. Isolation and Identification of Fungi

The plate count method was used to grow the fungi according to the method [1], where the medium of potato dextrose agar (PDA) was prepared according to the instructions mentioned on the medium box. Prepare 1 liter of the medium and stir well with heating until the complete dissolution then distributed to 250 ml in conical flasks with a capacity of 500 ml. Then the flasks containing the medium were sterilized with an autoclave at a temperature of 121 C and a pressure of 1.5 kg / cm for 15 minutes. After that, it was placed in a water bath at a temperature of 45 °C, then the antibiotic Chloramphenicol was added with concentration of 250 mg per liter. The sterile medium was poured into sterile plastic dishes with a diameter of 9 cm, and left to solidify in the laboratory. Then transfer 1 ml of the third

dilution of each sample to the plate and spread by the glass diffuser with three replicates for each sample.The inoculated dishes were incubated in the incubator at a temperature of 28 C for 5 days. The growing colonies were recorded on the plates according to the treatments of the samples. The average of the three replicates for each sample, were recorded.

2.4. Estimation of DON Toxin in Samples

DON toxin determination was carried out using a DON toxin kit from the Chinese company Shenzhen Lvshiyua Biotechnology Co.,Ltd. at the central laboratory at of Tikrit University as per the manufacturer's instructions included with the box. using the Eliza method.

2.5. The Procedure of Performing the Eliza Test

According to the test kit manufacturer's instructions, the toxin test kit was taken out of the refrigerator half hour before starting work and placed at room temperature. Sisty holes were selected from the drilling plate according to the number of samples and standard solutions, with three replications for each sample. 50 microliters were taken from each sample and its duplicates and placed inside the holes, then 50 microliters of the standard solution was added to each hole with light stirring after addition, then 50 microliters of the enzyme intended to bind to DON toxin was added for each hole, then moving the drilling plate with a light movement to ensure the homogeneity of the materials, after which the drilling plate was covered with special dark sheet, and incubated at a temperature of 25 C for half hour inside the incubator without lighting. after that the washing process of the pits was carried out automatically by the washing device of the ELISA device units. 4-5 times for 10 seconds for each wash and quantity250 µlof the wash solution provided with the test kit for each hole one during one wash. 50 µl of the base A material was added to each hole, then add 50 ml of base B material to each hole. with light stirring after each addition. Then incubate in the dark at room temperature after covering it for 15 minutes. A color will appear Blue in all the pits, then add 50 microliters of stop reaction solution to all the pits, then the color of the pits turns yellow. The pits are entered into the reader unit to find out the absorption values of all the pits, where the unit is regulated on the wavelength 450-630 nm. The reader unit sends a table of absorbance values for all pits to the printer unit.

3. Results and Discussion

3.1. Isolation and Identification of Fungi

The results of the detection of fungus contaminated samples of chips, corn flakes, popcorn, and miniatures on PDA as well as microscopic examinations showed that all samples under study were contaminated with fungi, note the Table (1.),and Figures: 1 & 2 . The results also showed a great dominance of *Penicillium spp*, as it was present in all samples by 100%, followed by *Aspergillus niger*, with 50%, as it was contaminated in nine samples. *Fusarium spp* appeared by 26%, since it was present in five samples, howver, the species ***Aspergillus tiresias*** found in three samples **.On** the hand, the species *Cladosporium herbarum* was found in only two samples. The Egyptian cornflakes were distinguished by containing three species of fungi, while the rest of the samples contained only two species in each sample, These results were consistent with [14], which confirmed the contamination of the local and imported chips product with fungi, but our results were to converged what was reached by [15] and agreed with what was reached by [16]. As well as , the resent results converged with what was found with [17], since it was mentioned that the most important fungi associated with corn grains were *Aspergillus, Fusarium, Penicillium, Mucor, Rhizopus* and *Trichoderma,* as the percentage of infection with *Aspergillus* reached 58%, followed by infection with *Fusarium* by 20.7%, as well as a large percentage of what was mentioned [18]. The present results are consistent with many studies, since the recorded species are storehouse fungi that have been monitored in various countries of the world [19]. These results are also consistent with [20], where the presence of *Penicillium, Aspergillus, Fusarium, Mucor* reached 40.2%, 37.4%, and 12%. .6%, 3.8%, respectively, and agrees with [21], as it indicated that the two genus *Aspergillus* and *Fusarium* are the most polluting fungi of chips and indomie.. It can explain the presence of Penicillium fungus, to a large extent, along with Aspergillus,

as it is a storage fungus. It also explains the presence of Fusarium, despite being a field fungus, which persists for months after storage [22].

Table 1. Presence of fungi contaminating corn chips and cornflex.

Contaminated Fungi	Sampl origin	Sapmle mark
Fusarium spp،Penicilliumspp	Egypt	Doritos
Aspergillus tiresias،Penicilliumspp	Iraq	BIG CORN
Fusarium spp،Penicilliumspp	Iraq	Salwan popcorn
Penicilliumspp Aspergillus niger	Iraq	Buffak
Fusarium spp،Penicilliumspp	Ukraine	Cornflex
Aspergillus niger،Penicilliumspp	Iraq	Cady
Penicilliumspp, Cladosporium herbarum	Iraq	Tortilla
Penicilliumspp, Aspergillus niger	Iraq	Dana
Aspergillus niger،Penicilliumspp	Iraq	Corn
Penicillium spp. Cladosporium herbaru Aspergillus niger	Egypt	Cornflex
Penicilliumspp, Aspergillus tiresias	Iraq	Jounna
Fusarium spp،Penicilliumspp	Iraq	Al-shami
Aspergillus tiresias،Penicilliumspp	Iraq	Salwan
Aspergillus niger،Penicilliumspp	Iraq	Ta'am
Aspergillus niger،Penicilliumspp	Malaysia	Cranchus
Aspergillus niger،Penicilliumspp	Iraq	Dalyia
Aspergillus niger، ،Penicilliumspp	Iraq	Hendrin
Fusarium spp،Penicilliumspp	Iraq	Panada

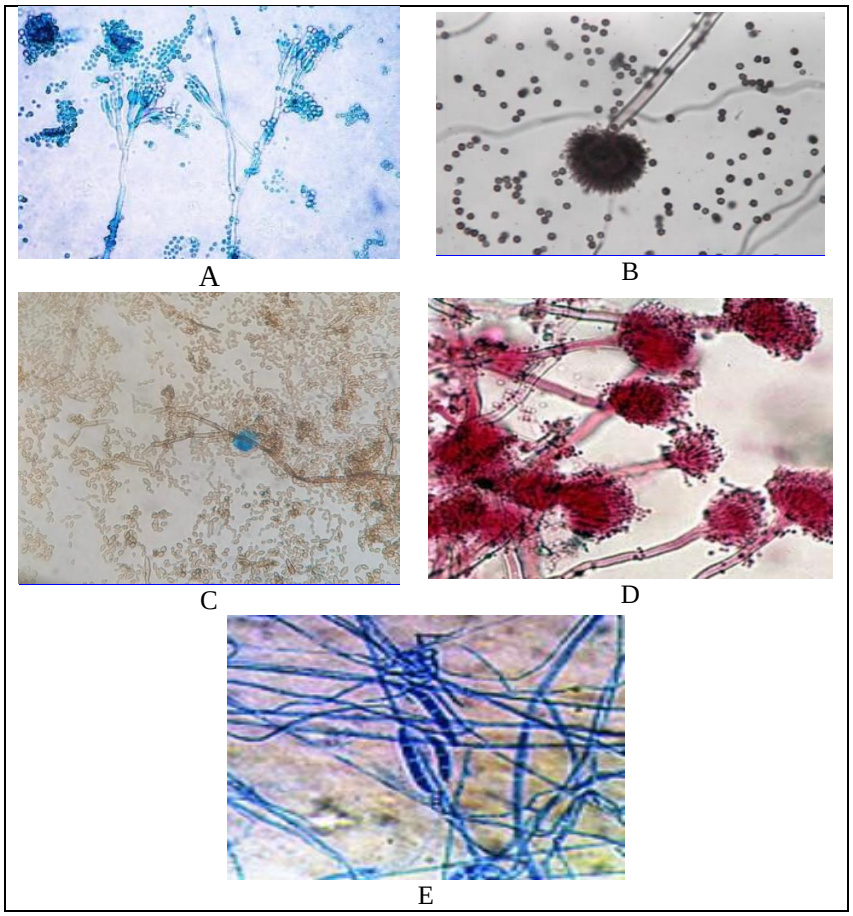

Figure 1. Microscopic images of fungal species found to contaminate corn products A . Penicillum ssp , B. Aspergillus niger , C. Clodoporium herbarum D . Aspergillus tiresias ,E. .Fusarium ssp.

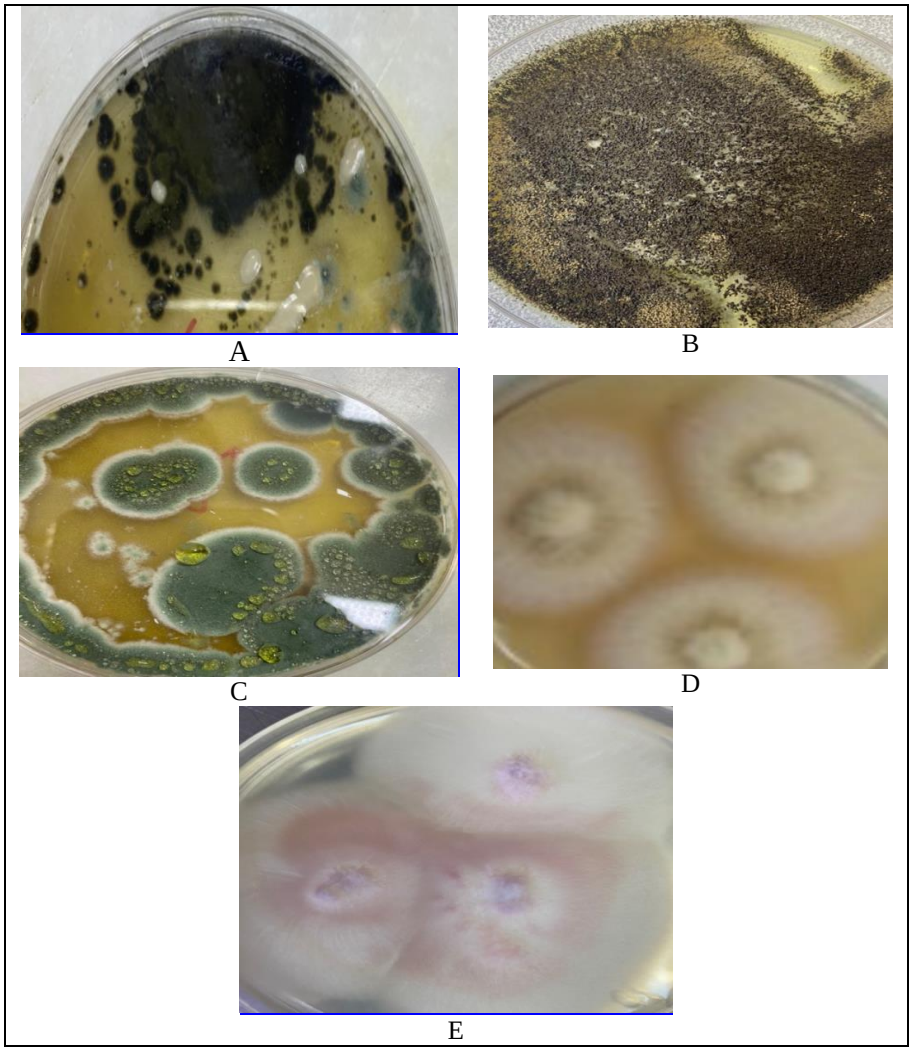

Figure 2. Pictures of colonies of fungi species that were found to be contaminated with maize products : A . Penicillum ssp, B. Aspergillus niger C. Clodoporium herbarum D . Aspergillus tiresias E. .Fusarium ssp.

3.2. Detection of DON Toxin Presence in Samples of Corn chips, Flakes and Popcorn

Table (2) shows the results of the detection of DON toxin in samples of chips and cornflakes by ELISA technique. It is noticed that all the samples under study were contaminated with DON toxin with different concentrations.The maximum concentration of the toxin was 3.69 mg/kg found in the Buffak chips sample(Iraqi), however, the lowest concentration was 1.57 mg/kg shown with Corn flex (Egypt), evethough it was contaminated with three species of fungi (Table-1) .There were another six sample ,the toxin concentrations were range between 3.60 as with Salwan popcorn sample and 3.00 as with Hendrin chips sample.. The rest Eleven samples showed the presence concentrations ranging between 1.57-2.80 mg/kg. These results agree with [9],which showed that all samples of chips and maize products were contaminated with DON at varying concentrations.

It is noticeable in Table (2) that the Buffak chips sample recorded the highest concentration of DON 3.69, although Fusarium fungus did not appear in the Buffak Chips sample, Table 1. This may be attributed to the poor storage of the yellow corn used in the manufacture of this product and its contamination with Fusarium (the producer of the DON poison during storage). So it is possible that the Fusarium fungus was killed during the manufacturing processes of the chips and the poison remained constant [23].

The results also showed in Table 2. That all the samples showed the presence of DON toxin in varying proportions, eventhough most of the samples did not show the presence of the fungus (*Fusarium*)

(table 1),which is responsible for the production of the DON toxin. This is may be due to the fact that the *Fusarium* fungus contaminated corn grains in the field or during the storage process and produced the toxin,and the manufacturing processes killed the fungus but did not destroy the toxin . All concentrations of DON toxin found in the samples were higher than the allowable average value of the range (0.3-2.0) mg/kg) in wheat and maize grains, and (0.75 mg/kg) in wheat flour, these values were approved by the European Union countries [10].

Table 2. DON concentration in the samples under study.

DON Concentration mg/kg	Sapmle origin	Sapmle mark
1.73	Egypt	Doritos chips
3.30	Irq	BIGCORN chips
3.60	Irq	Salwan popcorn
3.69	Irq	Buffak chips
1.54	Ukraine	Corn flex
1.62	Irq	Cady chips
3.40	Irq	Tortilla chips
3.50	Irq	Dana chips
2.80	Irq	ZER corn
1.57	Egypt	Corn flex
1.78	Irq	Joanna chips
3.25	Irq	Al-shami popcorn
2.63	Irq	Salwan Chips
2.40	Irq	Ta'am Chips
1.80	Malaysia	Crunchies chips
1.69	Irq	Dahlia chips
3.00	Irq	Hendrin chips
2.40	Irq	Popcorn Panda

Conclusion

- All samples included in the experiment were contaminated with fungi that produce dangerous mycotoxins.
- All samples were contaminated with deoxynivalenol.
- The maximum concentration of the toxin was 3.69 mg/kg found in the Buffak chips sample(Iraqi), however, the lowest concentration was 1.57 mg/kg shown with Corn flex (Egypt)
- All concentrations of DON toxin found in the samples were higher than the allowable average value that approved by the European Union countries.

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Bio-Physiological Impact of Treating Oxidative Stressed Local Rabbits with Aqueous Extract of Ginger Roots

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Abstract. This study was conducted in the field of the Technical Institute in bakraju /Sulaimaniyah Technical University, for the period from 2/4/2022 to 14/6/2022, which aimed to study the effect of hydrogen peroxide updated oxidative stress (0,5% with drinking water), and the ability of some antioxidants (aqueous extract of ginger tubers) (200 mg/kg) to affect the antioxidant status and performance bio-physiological characteristics of domestic rabbits, 48 male domestic rabbits aged 5-6 were distributed in 4 groups (12 rabbits/ group), and the treatment lasted for 42 days and was as follows : 1-control group 2-Group II: Hydrogen Peroxide (0.05%). 3-Group III: Aqueous extract of ginger tubers (200 mg/kg). 4-Group IV: Hydrogen Peroxide (0.05%) and Aqueous extract of ginger tubers (200 mg/kg). The results of the statistical analysis showed that the second group significantly affected all the studied biochemical indicators of blood serum and most of them were significant ($P \leq 0.05$) compared with the rest of the groups. It is represented by an increase in the level of glucose, cholesterol, triglycerides, enzymes AST, ALT , Malondialdehyde (MDA), low-density lipoproteins (LDL) and cortisol hormone And a decrease in the level of high-density lipoproteins (HDL) and glutathione (GSH). The aqueous extract of ginger tubers significantly affected all the studied biochemical and serum indicators and was mostly significant ($P \leq 0.05$) compared with the control group. It is represented by a decrease in the level of Cholesterol, Triglycerides, AST and ALT enzymes, Low-density lipoproteins, Malondialdehyde and Cortisol hormone. And an increase in the level of high-density lipoproteins and glutathione.

Keywords. Oxidative stress, Ginger Roots, Rabbits.

1. Introduction

Oxidative stress is a condition in which the concentration of free radicals and oxidants inside the body exceeds the concentration of naturally occurring antioxidants to get rid of them, and thus has an important role in the emergence and development of a number of diseases, including heart disease, diabetes, liver disease, arthritis, as well as cancer [1], neurodegenerative diseases [2], immune diseases [3] and many other ailments. Since free radicals attack the vital systems inside the body and break down nucleic acids and lipoproteins, they also oxidize fats, deform proteins and cause mutation mutations [4].

For this reason, recent studies have turned towards researching the role of antioxidants, especially those found in plants and medicinal herbs, which improve cellular performance and preserve cells from the occurrence of so-called oxidative stress [5]. Ginger *Zingiber officinale* is one of the many

medicinal plants used since ancient times in the treatment of many diseases , as well as being used as one of the common spices for food and drinks because of its distinctive flavour, where the ancient Chinese and Indians used it as a means of treating nausea [6]. Ginger was used as a folk remedy for weight loss, fat burning, a reducer of triglycerides and cholesterol in human blood [7], as well as an antioxidant [8,9]. Its oil also has a role in preserving deoxyribonucleic acid (DNA) deoxyribonucleic acid (RNA) from the oxidation process induced by hydrogen peroxide H_2O_2 due to the fact that it contains phenolic compounds and the amount of mineral and vitamin elements [10].
The current study aimed to find out the effect of oxidative stress induced by hydrogen peroxide, as well as to find out the effect of the aqueous extract of dry ginger tubers, which has antioxidant properties in the Bio-physiological performance of domestic rabbits, and its role in preventing or reducing the effect of oxidative stress induced by hydrogen peroxide.

2. Methods

Forty-eight adult rabbits of local male rabbits at the age of 5-6 months, which were obtained from local markets, were used in this study, and then the animals were randomly divided into four groups of 12 rabbits in each group. The animals were placed in cages, the dimensions of which ranged from one cage (2×2 M), which were prepared for breeding rabbits and under controlled environmental conditions of a temperature of 20-25 m and a lighting period (12 hours light - 12 hours dark).

2.1. Preparation of Ginger Extract

The ginger tubers were crushed well and then the ginger powder was grinded using an electric grinder, and (100) g of powder was taken and then mixed in (1000) ml of distilled water with a temperature of (25-20 m°) and then the mixture was left for a full hour in the horizontal vibrator at medium speed and then the mixture was left in order to settle for one hour and then it was filtered by WattMan Filter Paper No. (1) Then the filtrate was discarded through the use of the central ostracizer and at a speed of (3000) cycle / minute and for (15) minutes [11]. Then the extract was placed in the incubator at a temperature of 40 m° in order to obtain a dry powder of ginger extract, and then (2) g of ginger powder was taken and dissolved in (10) ml distilled water in order to obtain a concentration of (0.1) g/mL and this concentration was used as a storage (Stock solution) for the purpose of preparing the concentrations required in the research, which are 100 mg/kg, 200 mg/kg and 300 mg/kg.

2.2. Feeding Experiment Rabbits

The rabbits were fed based on a model feed intended for breeding rabbits to meet the growth and maintenance needs of the animal, as shown in Table (1), where feed and water were provided to the rabbits openly throughout the duration of the experiment.

Table 1. The components of the diet used in the experiment.

Primary Feed Material	%	Raw protein %
Wheat bran	47	7.50
Local barley	38	3.60
Soybean meal (44% protein)	10	4.40
Animal protein (50% protein)	2	1.0
Limestone powder	1	-
Salt	1.5	-
Vitamin and mineral mixture	0.5	-
Total	%100	%16.5

The amount of energy represented calculated in the BlackBerry was (2213) kilocalories/ kg of feed and the chemical analysis of the BlackBerry and the plants used in the study was carried out using the chemical analysis methods mentioned in [12].

2.3. Study Plan and Design of Experiments

The experiment was designed to study the effect of the aqueous extract of ginger tubers to reduce oxidative stress was tested using hydrogen peroxide H_2O_2 with drinking water on 48 adult males from the domestic rabbits were randomly divided into four groups, each group includes 12 rabbits. Including different biochemical blood parameters, represented by the concentration of serum enzymes,

represented by (AST) and (ALT) and the estimation of the concentration of cholesterol in the blood serum, triglycerides, high-density lipoproteins of cholesterol (HDL), low-density lipoprotein of cholesterol (LDL), serum glutathione level and serum Malondialdehyde.
The group of animals used in this experiment was divided into four groups and were as follows:

- The first group: (Control) negative control this group was treated with 1ml of distilled water.
- The second group: rabbits were treated with water with H_2O_2 added to it at a concentration of 0.5%.
- The third group: rabbits (200 mg/kg Rabbit/ Day) were dosed with the aqueous extract of the tubers of the ginger plant.
- The fourth group: rabbits were dosed (200 mg/kg rabbit/ day) with aqueous extract of ginger tubers in addition to (0.5%) of hydrogen peroxide by drinking water.

2.4. Blood Collection

Blood samples were collected at the end of each experiment, taking into account that the tests used in the study were performed on the experimental samples after the rabbits were evenly distributed among the experiment totals. Blood samples were collected from experimental animals after cutting off their feed for 12 hours, as the ear was the organ used in the blood collection process [13].

2.5. Statistical Analysis

The statistical analysis of the experiment data was carried out according to a complete randomized design CRD and using the ready-made statistical program [14] according to the following mathematical model:

$$Y_{ij} = \mu + S_i + e_{ij}$$

Where it represents:
Y_{ij} The viewing value of the studied attribute
μ Overall average
S_i The effect of the addition of ginger and hydrogen peroxide on the studied qualities
e_{ij} Experimental error that is distributed naturally and randomly
Duncan (1955) had been used [15], for the purpose of comparing averages with a significant level ($P \leq 0.05$).

3. Result and Discussion

The results of the statistical analysis in Table (2) showed that the treatment of rabbits with hydrogen peroxide significantly increased ($P \leq 0.05$) in the level of aspartate aminotransferase enzyme, alanine aminotransferase enzyme, blood cholesterol, triglycerides and the level of Malondialdehyde in the blood serum compared to all the remaining treatments, and decreased significantly ($P \leq 0.05$) in the the level of Glutathione compared to all groups. As for the treatment of rabbits with the aqueous extract of ginger tubers, it led to an impact on the values of all the studied biochemical indicators, as the results showed that it led to a significant increase ($P \leq 0.05$) of the Glutathione when compared with the rest of the treatments. A significant decrease ($P \leq 0.05$) in the level of Aspartate aminotransferase, Alanine aminotransferase, Cholesterol, Triglycerides and Malondialdehyde in comparison with the remaining treatments.
The addition of ginger with hydrogen peroxide (Group IV) led to the return of the levels of these indicators below to their level in the control group or better in varying proportions , where we note from table (2) that the fourth treatment led to a significant decrease ($P \leq 0.05$) in the level of aspartate aminotransferase enzyme, alanine aminotransferase enzyme, blood cholesterol, triglycerides, Malondialdehyde in the blood serum compared to only with hydrogen peroxide (0.05%) the second treatment. And led to an increase in the level of glutathione compare to the other treatments.

Table 2. The effect of the aqueous extract of the tubers of the ginger plant (*Zingiber officinale*) and hydrogen peroxide (0.05%) on some biochemical blood parameters.

Parameters / Treatments	Aspartate Amine transporter enzyme (IU/ L)	Alanine aminotransferase (IU/ L)	Total cholesterol (mg / 100 ML)	Triglycerides (mg / 100 ML)	Malondialdehyde (mmol/L)	Glutathi-one (mmol/L)
T1 Negative control (without treatment)	50.30±1.07 b	78.00±7.08 b	92.77±2.17 b	121.95±3.24 b	1.66±0.01 b	0.49±0.008 b
T2 H_2O_2 (0.05%)	62.20±3.98 a	114.12±6.13 a	114.40±2.91 a	160.32±1.41 a	2.09±0.12 a	0.36±0.02 d
T3 Ginger 200 mg/kg	40.25±1.87 c	45.42±4.85 c	75.50±3.88 c	93.80±1.31 c	1.40±0.03 c	0.59±0.01 a
T4 H_2O_2 (0.05%) + Ginger 200 mg/kg	46.15±1.80 bc	76.07±7.89 bc	97.35±4.59 b	125.35±2.69 b	1.76±0.03 b	0.43±0.01 c

* Different letters in the same column indicate that there are significant differences (P≤0.05) between the coefficients.

4. Discussion

The state of oxidative stress induced by hydrogen peroxide (Group II) led to a significant increase and at the level of probability (P≤0.05) at the level of both enzymatic AST and ALT compared with the control group. The reason for the significant increase in the concentration of both the AST and ALT enzymes may be due to the breakdown of most of the liver cell membranes as a result of oxidative stress resulting from lipid peroxidation and also an increase in the concentration of free radicals resulting from hydrogen peroxide, which consequently leads to the leakage of hepatic enzymes into the blood serum [16], and the reason for the increased production of free radicals that exceed the ability of antioxidants produced by the liver, especially glutathione, which consequently leads to hepatotoxicity [17].

On the other hand the decrease in the level of AST and ALT liver enzymes in the groups which treated with ginger aqueous extract may be due to the fact that ginger contains a high level of phenolic compounds as well as vitamin A and C, both of which are considered antioxidants, and this was indicated by [18]. Ginger, with this antioxidant effectiveness, scavenges free radicals such as the negative superoxide radical and, in turn, protects the liver from harmful factors and toxic damage caused by free radicals to liver cells, thereby stabilizing the cell membrane [19]. As for the reason for the significant increase in the concentration of cholesterol of the group treated with hydrogen peroxide in the blood serum may also be due to disorders in lipid metabolism due to oxidative stress and the occurrence of lipid peroxidation and unsaturated fatty acids, which causes inhibition of the secretion and excretion of steroid substances and bile salts, in addition to the occurrence of a group of disorders in the digestive and absorption processes in the intestine [20].

The dosing of rabbits with aqueous extract of ginger led to a significant decrease (P≤0.05) in the level of cholesterol and triglycerides in blood serum. [21] have also pointed out that substances have been isolated from ginger that have an effect on the vital cholesterol metabolism process in the liver. These substances increase the activity of the hepatic enzyme Cholesterol 7 - alpha-hydroxylase, which determines the rate of synthesis of bile acids, where it stimulates the conversion of cholesterol to bile acids and then the level of cholesterol decreases [22]. Many researchers have also shown that the active compounds in ginger 6-gingerol and 6-shogaol have a bioactive effect against cholesterol and thus reduce its level in the blood [23].

The reason for the significant decrease (P≤0.05) in the second group for the level of malonedialdehyde is due to an increase in oxidative stress, which leads to an increase in free radicals, and then due to an increase in the consumption rate of Glutathione, which is one of the most important non–enzymatic antioxidants in the removal of free radicals and their products, then it turns into its second ineffective form, which is Glutathione disulphide (GSSG), the sulfur group in the synthesis of glutathione acts as a good reducing agent, as a hydrogen atom blows easily due to the weakness of the bond between sulfur and hydrogen (S-H) and the binding force between (C–H) in free radicals, therefore, they protect cellular membranes from oxidative damage [24] .Perhaps the reason for the decrease in GSH is due to its consumption in the face of free radicals or reduced synthesis.

It is also noted from table (2) that the use of an aqueous extract of ginger has led to an increase in the level of glutathione as well as a decrease in the level of malondialdehyde. These results came in agreement with what the researchers [25] pointed out that the ginger plant is one of the plants that have antioxidant capacity because it contains a high percentage of reduced vitamin C, which is considered one of the most important water-soluble vitamins and antioxidants, as it is based on the removal of free radicals formed continuously, which works to provide protection from their effects. Or perhaps the improvement in the antioxidant status is due to the fact that ginger contains many active substances, including shagol, camphene and zingiparol, which have a key role in the process of protecting organism cells from oxidative damage caused by the presence of free [26]. Also, the presence of volatile oils in ginger has a significant role in protecting against free radicals [27].

As for the addition of ginger with hydrogen peroxide, this led to the removal or prevention of the effect of oxidative stress, as it improved the antioxidant status, as evidenced by the significant increase (P≤0.05) of the level of glutathione compared to the hydrogen peroxide group, as well as the significant decrease of the level of malondialdehyde compared to the hydrogen peroxide group. This is due to the antioxidant capacity of ginger [26].

Conclusion

Based on the results which obtained the following conclusions:

- Oxidative stress with hydrogen peroxide in male rabbits led to a decrease in the level of antioxidants, as the level of glutathione decreased significantly in the blood serum of rabbits exposed to oxidative stress compared to normal rabbits, as well as an increase in the level of malondialdehyde.
- Oxidative stress with hydrogen peroxide in male rabbits caused negative changes, represented by an increase in the level of cholesterol, triglycerides , Aspartate Amine transporter enzyme and Alanine aminotransferase

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Effect of eCG and hCG Injections on Testicular Dimensions and Sex Cells in Iraqi Shami Bucks

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Abstract. The current study aimed to show the effect of hormonal treatment with eCG and hCG on spermatogonia in Iraqi Shami buck. 12 Shami buck of 1.5-2 years old. Bucks were divided into four groups, (T_1) left without treatment as control group, (T_2) was injected with hCG (250 IU/ Animal), (T_3) was injected with eCG (250 IU/ Animal) and (T4) fourth group was injection were injected with hCG and eCG (250 IU/ Animal). The injection was done periodically once every two weeks. The animals were slaughtered at the end of the experiment. Diameter of testis (cm), Testicular circumference (cm), Length of testis (cm), Testis weight (g), Size of testis (cm^3), Germ cell layer, Number of spermatogonia cells, Seminiferous tubule diameter, Sertoli cells and Number of Leydig cells were measured. The results indicated that, the was a significant effect ($P<0.05$) of the eCG and hCG hormone on the Diameter of testis, Testicular circumference and Testis weight, where both treatments T_3 and T_4 were superior as compared with treatment T_2 and T_1, respectively, and T_4, T_3 and T_2 outperformed ($P<0.05$) on treatment T1 significantly ($P<0.05$) in Length of testis and Size of testis. Also, number of Sertoli cells and Leydig cells increased ($P<0.05$) in T_4, T_3 and T_2 than other treatments. While Spermatogonia, Germ cell layer and Seminiferous tubule diameter increased significantly in the treatments in T_4 and T_3 treatments than other treatments. We conclude from this study that eCG and hCG injections have beneficial effect on spermatogonia and testicular dimensions in Shami buck.

Keywords: eCG and hCG, Testicular dimensions, spermatogonia, Shami buck.

1. Introduction

Goats are a seasonal breeder with multiple estrus, which is affected by environmental factors, especially the length of the daily lighting period of the seasons, as both males and females enter a period of sexual silence period of (3-6) months [1]. These changes are reflected in the reproductive and anatomical characteristics of the male reproductive system, in addition to changes in the quantity and quality of semen in males [2-3]. Several studies have been conducted to improve the reproductive efficiency of buck, including hormonal and vitamin treatments [4-5].

Equine chorionic gonadotrophin (eCG) is a complex glycoprotein characterized by both FSH and LH activity but with a higher carbohydrate content [6-7]. Its main characteristic is the long half-life [8]. Human Chorionic Gonadotropin (hCG) The glycoprotein are consists of a and f3 subunits. The a subunit of hCG is similar to the a subunits of animal and human LH. hCG is primarily luteinizing, luteotropic and has little FSH activity. The syncytiotrophoblastic cells in the primate placenta

synthesize hCG of the placenta of primates; It is found in the urine after 8 days of pregnancy, as well as in the blood [7].
The eCG also to stimulate follicular growth, leading to a higher ovulation, lambing, twinning rates and improved fresh, frozen-thawed semen quality and stimulated the secretion of testosterone in and out-breeding season [9- 10- 11]. Therefore, the present study aimed to define the effect of hormonal treatment with eCG and hCG on spermatogonia in Iraqi Shami buck.

2. Materials and Methods

12 Iraqi Shami bucks of 1.5-2 years old and 37-52 kg body weight were used in the current study, placed in the filed of College of Agriculture/ University of Diyala, during the period from 20/6/2020 to 20/11/2020. Bucks were divided into four groups, (T_1) first group lifted without treated as control group, (T_2) second group was injected with hCG (250 IU/ Animal), (T_3) third group was injected with eCG (250 IU/ Animal) and (T_4) fourth group was injection were injected with hCG and eCG (250 IU/ Animal). The injection was done periodically once every two weeks.
The animals were slaughtered at the end of the experiment. The Testes were taken and placed in a tray containing NaCl (0.9%) physiological solution, the length and diameter of the testes were measured by means of a vernier, and the testes volume was measured by displaced water. A large capacity of 500 ml, and the displaced water is placed inside a graduated cylinder to measure its volume, measure the weight of the testes by an electronic sensitive scale, and the circumference of the testes with the included measuring tape. Automatic microtome tissue section was counted according to [12]. SPSS program (2012) [13] were used analyzed of Data. Duncan multiple range test were used to compare the significant difference means[14].

3. Results and Discussion

Table-1 showed the hCG and eCG hormone injection treatments on the diameter, circumference, length, weight and size of the testis. The treatment T_4 and T_3 as compared to the T_2 and T_1 in the diameter of testis, testicular circumference and testis weight, respectively, While the T_4, T_3, and T_2 ($p < 0.05$) as compared to the treatment T_1 in the length of the testis and size of the testis, respectively.

Table 1. Effect of hCG and eCG on testicular dimensions of male Shami bucks.

Treatment	Diameter testis (cm)	Testicular circumference (cm)	Length of testis (cm)	Testis weight (g)	Size of testis (cm^3)
T_1	4.22 ± 1.24 c	9.27 ± 1.22 c	7.23 ± 0.13 b	88.22 ± 1.61 c	80.15 ± 1.45 b
T_2	5.21 ± 0.11 b	12.45 ± 1.24 b	8.18 ± 0.18 a	100.41 ± 2.17 b	90.12 ± 1.32 a
T_3	6.00 ± 1.33 a	13.44 ± 0.33 a	8.21 ± 0.14 a	110.77 ± 3.38 a	90.00 ± 1.22 a
T_4	6.14 ± 0.14 a	13.22 ± 0.21 a	8.33 ± 0.17 a	113.56 ± 2.21 a	92.12 ± 0.14 a

a, b and c values with different superscripts within same column are significantly different ($P<0.05$). Mean values ± S.E.M.

The increase in testicular dimensions is due to the effect of injections of the hormones hCG and eCG, that acts similarly to the hormones of FSH and LH [15- 16- 17]. It affects the activity and development of the testis [18].
Also, the number of Sertoli cells leads to an higher in the size of the testis [19]. The eCG stimulates the ICSH receptors, which increases the fluid content in the testis, by increasing the blood flow to the testis and the permeability of the capillaries [20].
Table-2 showed the effects of hCG, eCG hormone injections on the number of Sertoli cells, with T_4, T_2 and T_3 treatments outperforming T_1 treatment. The numbers of Leydig cells in the T_4, T_2 and T_3 treatments were superior than the T_1 treatment, and the numbers of Spermatogonia cells in the T_4 and T_3 treatments were superior than those in the T_2 and T_1 treatment. The thickness of the germ cell layer followed the same path as the number of Spermatogonia cells, where treatment T_4, T_3 as compared to treatment of T_2, T_1. As for the Seminiferous tubule diameter, treatment T_4, T_3 as compared to the rest of the T_2, T_1.

Table 2. Effect of hCG and eCG on testicular cells of Shami bucks.

Treatment	Sertoli cells	Leydig cells	Spermatogonia	Germ cell layer	Seminiferous tubule diameter
T_1	23.22 ± 0.32 b	22.11 ± 0.44 b	18.35 ± 0.53 c	42.55 ± 0.11 c	125.27 ± 5.16 c
T_2	36.52 ± 1.23 a	28.51 ± 1.72 a	27.15 ± 0.14 b	47.38 ± 0.32 b	177.33 ± 7.22 b
T_3	35.96 ± 0.23 a	30.32 ± 0.35 a	30.13 ± 0.14 a	53.18 ± 0.44 a	207.22 ± 7.18 a
T_4	36.44 ± 0.42 a	32.27 ± 1.39 a	31.11 ± 0.17 a	54.26 ± 0.48 a	210.44 ± 8.18 a

a, b and c values with different superscripts within same column are significantly different ($P<0.05$). Mean values ± S.E.M.

Studies have shown that injections of hCG leads to increased the secretion of testosterone, that acts on the testis development and maintenance of primary and secondary male sex characteristics. The injection of hCG directly affects the ICSH, which leads to an increase in Leydig cells. ICSH stimulates the production of testosterone by the Leydig cells, testosterone stimulate spermatogenesis and water absorption of the testis and seminal vesicles [21- 22]. Also, the increase in the numbers of Leydig cells is due to the effect of the eCG hormone in increasing the numbers of these cells [23]. The reason that higher in Sertoli cells is due to the increase in testosterone, which acts to complete the growth of spermatogonea in the seminiferous tubule, and the injection of hCG leads to an increase in the level of testosterone [24- 25]. Thus, it helps in the process of formation and division of germ cells and increase their numbers[26]. The ICSH stimulates the growth and development of the interstitial cells and their transformation into Leydig cells that secrete testosterone and produce the necessary enzymes for this through its association with the receptors on the outer surface of the cell, while the SSH stimulates the growth and widening of the seminiferous tubules, and an increase in Sertoli cells inside the seminal tubule and the germ layer [17- 19]. The eCG also stimulates the activation of seminiferous epithelium and Leydig cell function, and increases the size of the seminiferous tubule. It increases the diameter of the seminiferous tubule and activates the Spermatogenic cells [16]. Also, the injection of hCG hormone into the Hypophysectomy animals led to the re-formation of Leydig cells[27].

Conclusion

It is concluded that injections of hCG and eCG (250 IU/ Animal) improved diameter of testicular, testicular circumference, length, weight and size of the testis. Also increased numbers Sertoli cells, Leydig cells, Germ cell layer, Spermatogonia and Seminiferous tubule diameter in Iraqi Shami buck.

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Isolation and Identification of Yeasts from Strawberry and Evaluation of Their Efficiency in Inhibiting the Pathogenic Fungus *Botrytis cinerea* Caused the Gray Rot Disease

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Abstract. 16 yeasts were isolated from the various parts of the cultivated strawberry in some regions of Salah Al-din province , which included Samarra, Al-Alam, Balad, and Tikrit. 10 yeasts isolates showed antifungal activity against Botrytis cinerea, while 5 isolates did not show any antifungal activity. The results showed that the cell suspension of Y-9 isolate achieved the highest antifungal activity with inhibition zone reached 1.1 cm with a significant superiority over the other isolates, followed by the Y-2 and Y-5 isolates, in which the inhibition zones reached 0.9 cm, while the isolates Y-1, Y-4, Y-7, Y-11, Y-12, and Y-14 did not record any antifungal activity. The yeast isolate Y-9 filtrate also achieved a significant superiority in its antifungal activity compared to the other isolates, in which the inhibition zones reached 1.3 cm, followed by 1.2 and 1.1 cm in the Y-5 and Y-2 isolates, respectively. The activities of the chitinase and the β-glucanase were estimated for the yeast isolates that showed the highest inhibition against the pathogenic fungus B. cinerea. The results showed that all the selected yeast isolates have β-glucanase, the isolate Y-9 achieved the highest enzymatic activity of the β-glucanase reached 1.82 units/ml, followed by Y-5 and Y-3 with enzymatic activity of 1.67 and 1.51 units/ml, respectively. The isolate Y-9 also recorded the highest activity of the chitinase reached to 3.44 units/ml followed by the isolate Y-5 with a chitinase activity of 3.12 units/ml. The yeast isolates that showed the highest inhibition against the pathogenic fungus were diagnosed at the species level using the analysis of the nucleotide sequences of the 5.8S rRNA gene, the nucleotide sequences of this gene matched by 99.19-99.51% with the yeasts registered in the World GenBank. Yeast species were recorded for the first time as Iraqi isolates in the world Gene Bank; *Rhodotorula* glutinis isolate Has.AA-44, *Rhodotorula* glutinis isolate Has.AA-47, *Rhodotorula* mucilaginosa isolate Has.AA-45 and *Saccharomyces* cerevisiae isolate Has.AA-46. and *Zygosaccharomyces* rouxii isolate Has.AA-48 under the accession numbers OQ108347.1, OQ108351.1, OQ108369.1, OQ108376.1, and OQ108370.1, respectively.

Keywords. Antifungal yeasts, Strawberry, Pathogenic fungus *Botrytis cinerea*, Biological control, Nucleotide sequence analysis, Molecular diagnosis.

1. Introduction

Strawberry is an important and widespread fruit in the world, it belongs to the Rosaceae family and the genus Fragaria, which includes 45 wild and cultivated species. There are more than 200 different

cultivars distributed in Europe, Asia and North America [1]. The global production of strawberries in 2020 amounted to 8.88 million tons in an area of 3.96 thousand hectares [2]. The strawberry crop is characterized by good nutritional value and good flavor, as it contains many nutrients such as proteins, carbohydrates, fats, calcium, magnesium, phosphorus, potassium, copper, and zinc, in addition to vitamin C, thiamine, and riboflavin [3]. Strawberries are affected by many plant pathogens including fungi, bacteria, viruses, and nematodes. The most economical pathogen for strawberries is fungi, which can infect all parts of the plant and cause it to rot and die [4]. The fungus *Botrytis cinerea* is the main pathogen of strawberries in the world, which leads to great economic losses, as it causes gray rot disease on all parts of the plant. This disease occurs not only in the field, but also during strawberry storage, transportation and marketing [5]. In the humid conditions, *B. cinerea* causes a loss of more than 80% of strawberry flowers and fruits [6], Due to the negative impact of fungicides on the environment and the development of the resistance pathogens to the fungicides as a result of their continuous use, as well as the threat to human health and all the ecosystem [7,8], Biological control has become an urgent necessity as it is safe and environmentally friendly as well as for their anti-pathogen effect [9]. In biological control, many microorganisms were found capable of protecting plant and fruit from fungal diseases, including bacteria, filamentous fungi, and yeasts [10].

Yeasts have an advantage among microorganisms because their nutritional requirements are rather simple, they can be easily produced, not harmful to humans, the environment, or the host fruit, and the target organisms are unlikely to generate resistance [11].Yeasts colonize the tissues of plants and have multiple benefits for their hosts, including disease suppression, however, among these mechanisms: mycoparasitism through the secretion of lytic enzymes [12], competition for nutrients and space [13], production of siderophores, toxic metabolites, and volatile compounds [14], as well as the induction of systemic resistance in plants [15]. Also, the secretion of enzymes that lyse the walls of pathogens such as chitinase, glucanase, protease, and lipase play an important role in inhibiting the growth of pathogens [16].

Due to the importance and prevalence of the gray rot disease caused by the fungus *B. cinerea* on strawberries, and due to the lack of studies on the use of yeasts in the biological control program , the present study aimed to isolate and characterize yeasts from strawberries visually and molecularly, in addition to evaluate their efficiency in inhibiting the pathogenic fungus *B. cinerea*.

2. Materials and Methods

2.1. Yeast Isolation

Samples of strawberry plants were collected from the regions of Samarra, Al-Alam, Balad, and Tkrit within Salah Al-din province, these samples were transferred to the laboratory, washed well using distilled water for 5 minutes, and superficially sterilized by 3% sodium hypochlorite for one minute. The plant pieces were washed with distilled water for removing the chlorine, then they were spread on sterile filter papers to absorb excess water, finally transferred to petri dishes containing Yeast extract Peptone Dextrose Agar (YPDA).

2.2. The Pathogenic Fungus Botrytis Cinerea

An isolate of the pathogenic fungus *B. cinerea* (which identified phenotypically and molecularly) was used from the laboratory of the Department of Plant Protection - College of Agriculture - University of Tikrit [17].

2.3. Morphological Identification of the Yeast Isolates

The isolated yeasts were initially identified by the texture, colour, and shape of the growing colonies, as well as the phenotypic shapes and dimensions of the yeast cells after examination with a compound microscope.

2.4. Preparation of the Yeast Cell Suspension and Filtrates

Yeast isolates were grown (separately) in a liquid medium,YPD Broth, by transferring a swab of purified yeasts using inoculation loop, and incubating at a temperature of 25 ° ± 2 for (2-3) days.

Centrifuged at 5,000 rpm for 10 minutes, then withdraw the filtrate using a medical syringe and sterilize the filtrate using the 0.22 µm millipore filter. The precipitate (represents yeast cells) was collected after suspended with sterile normal saline, then the yeast cells number was adjusted to 10^8 yeast cells / ml using a counting slide.

2.5. Antagonism Between Yeast Cell Suspensions and Their Filtrates Against the Pathogenic Fungus B. Cinerea

Antagonism test between the pathogenic fungus *B. cinerea* and the yeasts suspension was performed by double cultivation in a Petri dish containing the culture medium YPD, briefly, a piece of the colony of the pathogenic fungus *B. cinerea* was taken through a cork borer with a diameter of (0.5) cm and placed in the center of the plate containing the YPDA. The medium was plotted with a line of yeasts using a sterile loop at a distance of 3 cm from the fungus disc. The plates were incubated at 25 ° C for 2-3 days, after which the inhibition zone was measured by a digital ruler between the line yeast growth and the edge of the fungal colony. The same method was used to the antagonism test between the yeast filtrate and the pathogenic fungus *B. cinerea*, except that the layout was replaced by the filtrate instead of the yeast cells [17].

2.6. Effect of Cells and Filtrate of Yeast Isolates on Strawberry Fruits

The effect of yeasts isolates were tested on the fresh and intact strawberry fruits obtained from the local markets, as they were surface sterilized with sodium hypochlorite 3% for one minute, then the fruits were washed with distilled water, and spread on sterilized filter papers to absorb excess water. 20 fruits were used in each treatment. The fruits were dipped for one minute with yeast cell suspensions and their filtrates separately, then incubated at laboratory temperature for 5 days. The degree of damage to the fruits was calculated through the appearance of symptoms of tissue laceration and color variation, according to the following equation:

Infection rate = number of damaged fruits / total number of fruits x 100

2.7. Estimation of the Chitinase and β-glucanase Activities for the Highly Antagonistic Yeasts

2.7.1. Enzyme Extraction

Crude enzymes were extracted from yeast isolates that recorded the highest antipathy against pathogenic fungus and did not affect strawberry fruits after growing them in the medium of the minimum mineral medium that modified by [18], briefly; 5 g of pure chitin was added instead of ammonium nitrate and glucose to extract the chitinase, while to extract the glucanase, 5 g of pure β-glucan was added instead of glucose, the media were incubated at 25 °C for 5 days, then filtered using Whatman No.1 filter paper, and centrifuged at 5000 rpm for 10 min, finally, the filtrate was withdrawn which represents the crude enzymes source.

2.7.2. Chitinase Assay

Chitinase was determined according to [19], where 0.5 ml of the crude enzyme was added to 0.5 ml of the chitin solution (1%) for each yeast isolates separately, and incubated in a water bath for one hour at 35 ± 1°C, then 1 ml of DNS solution was added to mixture, and boiled in the water bath at 100 °C for 5 minutes, after that cooled and the absorbance was measured using a UV spectrophotometer - at a wavelength of 540 nm. The enzyme unit (unit. ml^{-1}) was defined as the amount of enzyme required to release 1 micromol of the substrate (chitin) per ml of filtrate at one minute.

2.7.3. β-glucanase Assay

This enzyme was determined based on [20] by adding 0.5 ml of crude enzyme to 0.5 ml of β-glucan solution (1%) for each yeast isolate separately, then the same steps of chitinase assay was carried out for β-glucanase assay. The enzyme unit (unit. ml^{-1}) was defined as the amount of enzyme required to release 1 micromol of the substrate (β-glucan) per ml of filtrate at one minute.

2.8. Molecular Identification of the Highly Antagonistic Yeasts

The nucleotide sequence analysis of the ITS1-ITS4 region of the 5.8S rRNA gene, was used for the identification of the yeast isolates at the species level. DNA was extracted from the yeasts by taking (100) mg from a newly developed colony (3 days old) of each yeast isolate separately. DNA was extracted using ZR Fungal/ Bacterial/ Yeast DNA mini prep TM kit (provided from the American company ZR) according to the manufacturer's instructions. Electrophoresis was carried out using a 1.5% agarose gel, after that the genomic DNA bands were exposed using ultraviolet light (302 nm) after being stained with Intron red stain (Korea) then, the resulting bands photographed using a digital camera.

A polymerase chain reaction (PCR) was conducted to amplified the ITS region within the 5.8S rRNA gene. The Maxime PCR PreMix Extraction Kit (i-Taq) 20µl rxn (Cat. No. 25025) with the primers; Forward 5′- TCCGTAGGTGAACCTGCGG -3′ and Reverse 5′ TCCTCCGCTTATTGATATGC-3′ were used for the amplification [21].

The components of the reaction mixture with their concentrations were at final volume of 25 µl as follows: Taq PCR PreMix at a concentration of 5 µl, Forward primer at a concentration of 10picomol/ml (1 µl), Reverse primer at a concentration of 10 picomol/ml (1 µl), DNA at a concentration of 1.5 µl, Distill water (16.5) µl). the reaction program included 37 cycles, and each cycle included the following: (i) Initial denaturation at a temperature of 95 ˚C for 5 minutes (ii) Denaturation at a temperature of 95 ◦C for 45 seconds (iii) Annealing stage at a temperature of 95 C for 45 seconds. 58 ◦C for 45 sec (iv) First extension stage at 72 ◦C for 45 sec (v) Second extension stage at 72 ◦C for 7 min. Thermocycler Apparatus (Applied Biosystem Gene-amp PCR System 9700) was used for gene amplification process. The PCR product was separated using agarose gel electrophoresis (1.5%), then PCR products were demonstrated using UV light with a wavelength (302 nm) after treatment with Intron Korea red stain.

2.9 Nucleotide sequencing analysis

The nucleotide sequences of the PCR amplified gene were determined after obtaining the 5.8S rRNA amplifications directly by sending a volume of 25 µl of PCR product and a volume of 10 µl (10 picomole concentration) of each primer to the Korean company Biotechnology Lab (Applied Biosystem 3730XL, DNA Sequencer device used). The results were compared using a web-based computer program (the basic in-situ nucleotide sequence search tool). Basic Local Alignment Search Tool (BLAST) with the database at the National Center for Biotechnology Information (NCBI), which matches the nucleotide sequences of the studied genes with respect to the yeast isolates placed in the search and determined their species according to the match in the aforementioned database.

2.10 Statistical analysis

Statistical analysis of the resulted data were carried out by analysis of variance using the program (SPSS). The means were compared according to the Least Significant Deference test (L.S.D.) at the level of probability 0.05 [22].

3. Results

3.1. Isolation of Yeasts from Strawberry Fruits

Sixteen isolates of yeasts were isolated from different parts (roots, stem, leaves and fruits) of strawberry grown in some districts of Salah Al-din province, which included (Samarra, Al-Alam, Balad and Tikrit) as shown in Table (1). The results showed that 8 yeast isolates were isolated from strawberry fruits, 4 isolates from leaves, 3 isolates from roots and one isolate from the stem.

Table 1. Sources of yeast isolates.

Isolate code	Strawberry part	Districts
Y-1	Leaves	Samarra
Y-2	Fruits	Samarra
Y-3	Fruits	Samarra
Y-4	Leaves	Al-Alam
Y-5	Roots	Al-Alam

Isolate code	Strawberry part	Districts
Y-6	Fruits	Al-Alam
Y-7	Leaves	Al-Alam
Y-8	Roots	Balad
Y-9	Fruits	Balad
Y-10	Fruits	Balad
Y-11	Leaves	Balad
Y-12	Stems	Balad
Y-13	Fruits	Tikrit
Y-14	Roots	Tikrit
Y-15	Fruits	Tikrit
Y-16	Fruits	Tikrit

3.2. Antagonistic Activity of the Isolated Yeasts Against the Pathogenic Fungus B.cinerea

3.2.1. Effect of the Yeast Cell Suspension

The results of figure (1) showed the significant differences in the antagonistic activity among the tested yeasts, 10 isolates showed antagonistic activity against the fungus *B. cinerea*, while 6 isolates did not show any antagonistic activity, the results also showed that isolate Y-9 achieved the highest antagonistic activity with an inhibition zone of 1.1 cm (with a significant superiority over the other yeasts), followed by isolates Y-2 and Y-5 with an inhibition zone of 0.9 cm. On the other hand, isolates Y-10, Y-13 and Y-15 achieved the lowest level of inhibition, which reached 0.1 cm, while the isolates Y-1, Y-4, Y-7, Y-11, Y-12, and Y-14 did not record any antagonistic activity. Figure (2) showed the highest antagonism recorded by yeast isolate Y-9 against fungus *B cinerea*.

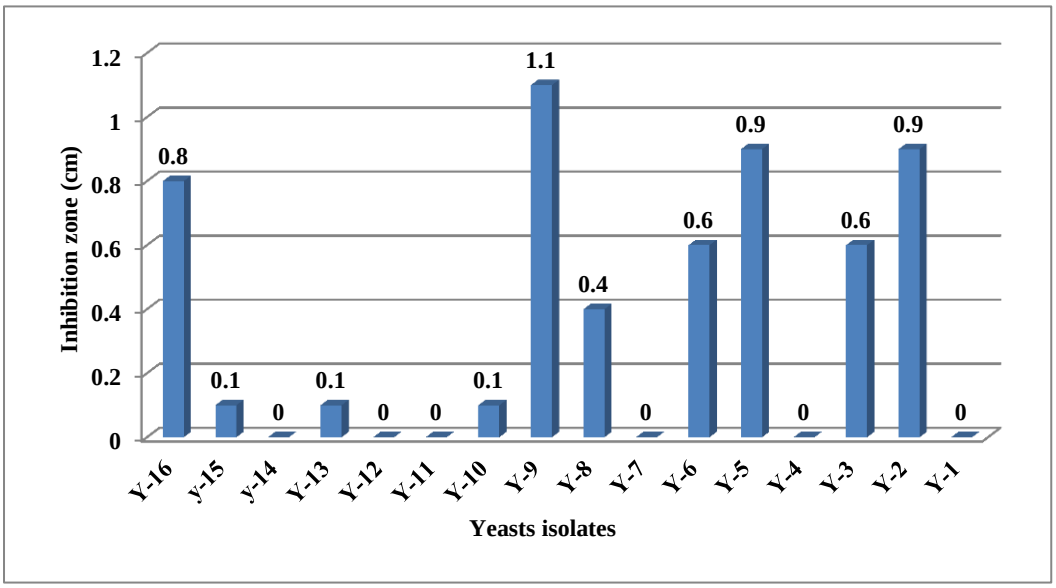

Figure 1. Effect of the yeast cell suspension on the inhibition of the pathogenic fungus *B. cinerea* (LSD 0.05 ; 0.05).

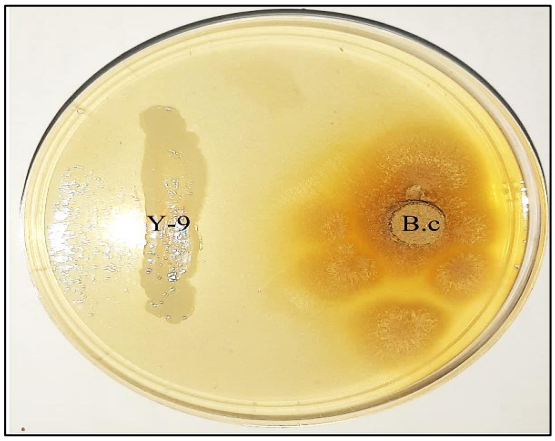

Figure 2. Effect of Y-9 yeast cell suspension in inhibiting the pathogenic fungus *B.cinerea*.

3.3. Effect of the Yeast Cell Filtrate

The results of figure (3) showed that the filtrate of isolate Y-9 had a significant superiority in its inhibitory activity (compared to the filtrate of other isolates) with an inhibition zone of 1.3 cm, followed by 1.2 and 1.1 cm in the filtrate of isolates Y-5 and Y-2, respectively, compared to the lowest antagonism with an inhibition zone of 0.3 cm by the isolates Y-10 and Y-13, while the filtrate of isolates Y-1, Y-4, Y-7, Y-11, Y-12 and Y-14 did not achieve any antagonistic activity against the pathogenic fungus *B. cinerea*.

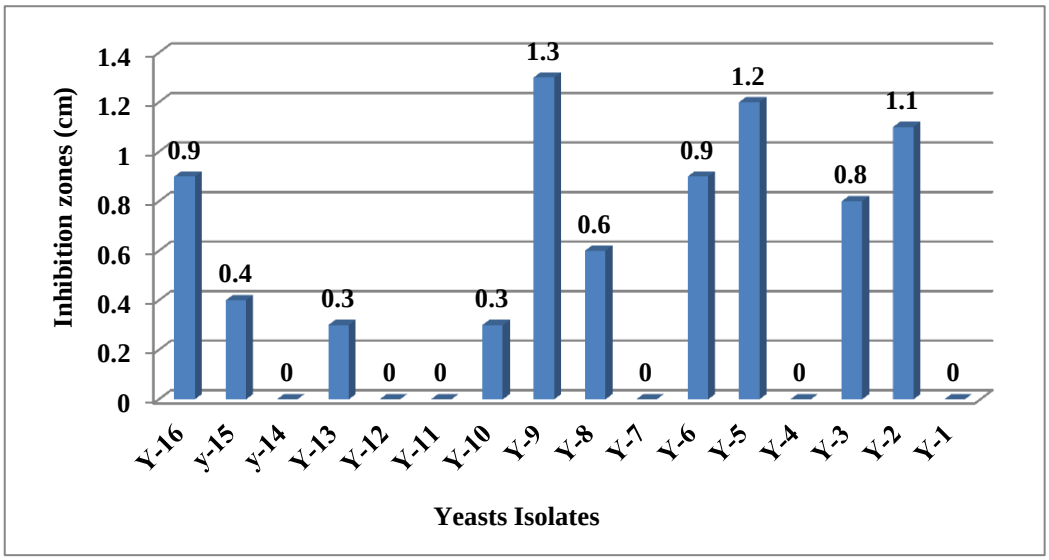

Figure 3. Effect of the yeast cell filtrate on the inhibition of the pathogenic fungus *B. cinerea* (LSD 0.05 ; 0.07).

3.4. Effect of Cell Suspension on the Infection of Strawberry Fruits

Figure (4) showed the effect of yeast isolates on infecting strawberry fruits. The results showed the infection of strawberry fruits achieved by nine isolates (Y-2, Y-4, Y-6, Y-8, Y-10, Y-11, Y-12, and Y -13 and Y-16). Both isolates (Y-10 and Y-16) showed the highest infection rate of strawberry fruits, reached to 59.65 and 52.05%, respectively, compared to the lowest infection rate 4.54% by the isolate Y-6, while the isolates (Y-1, Y-3, Y-5, Y-7, Y-9, Y-14 and Y-15) did not record any change in strawberry fruits.

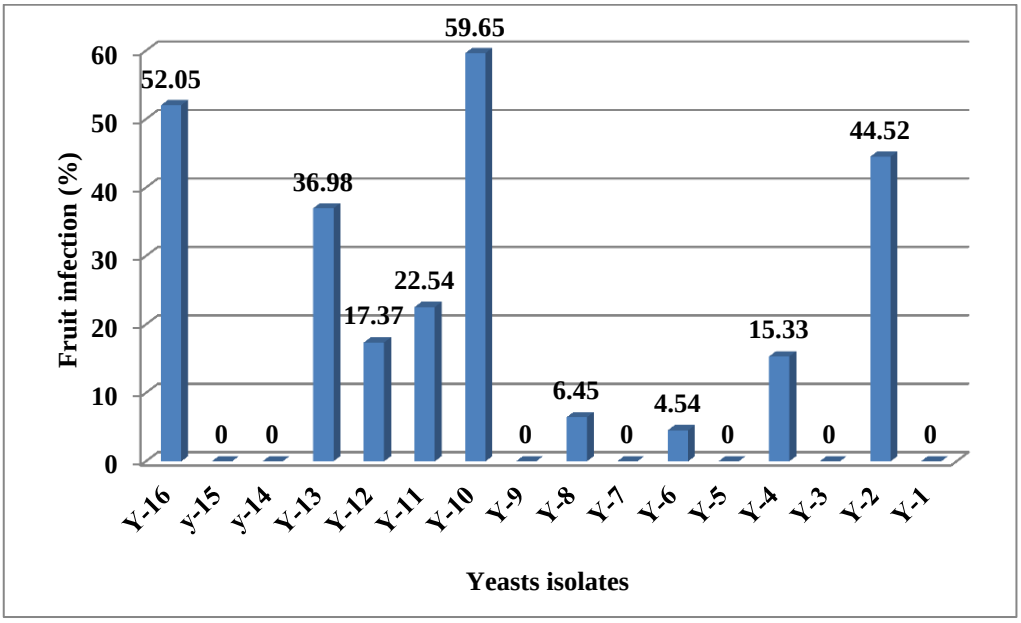

Figure 4. Effect of yeasts cell suspension on the infection of strawberry fruits (LSD 0.05; 2.36).

3.5. Effect of Yeast Filtrate on the Infection of Strawberry Fruits

Figure (5) showed the effect of yeasts filtrates on infecting strawberry fruits. The results showed that strawberry fruits were infected by eight isolates (Y-2, Y-4, Y-6, Y-10, Y-11, Y-12, and Y- 13 and Y-16). The isolates (Y-10 and Y-16) showed the highest infection rate of strawberry fruits, reached to 63.69 and 63.33%, respectively, while the isolates (Y-1, Y-3, Y-5, Y-7, Y-8, Y-9, Y-14 and Y-15) did not record any change in strawberry fruits.

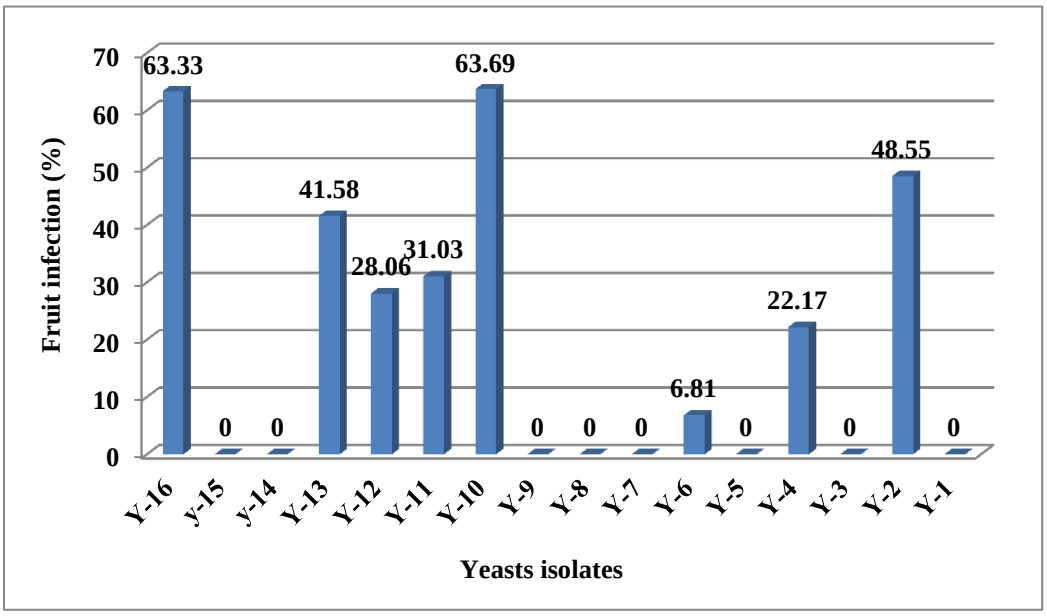

Figure 5. Effect of yeast isolates filtrates on the infection of strawberry fruits (LSD 0.05; 3.51).

3.6. Preliminary Diagnosis of Yeast Isolates with the Highest Inhibition of the Pathogenic Fungus

The macroscopic and microscopic phenotypic characteristics of the yeast isolates that recorded the highest inhibition of the pathogenic fungus were studied. Isolate Y-6, isolated from the strawberry fruits from the Al-Alam district, was characterized by its pink colonies, gelatinous texture, and round shape, while its cell shapes were spherical-oval, with dimensions of 2-3×5-6 µm (Fig. 6-A). As for the isolate Y-3 isolated from Samarra district, their colonies were distinguished by their light orange color,

mucous texture, and round shape, while their cell shapes were ovoid-elliptical, with dimensions of 1.5–4 × 4–12 µm (Fig. 6-B), while, isolate Y-8, which was isolated from the strawberry roots from Balad district, was distinguished by its white-creamy colonies, gelatinous texture, and round, irregular shape. Its cell shapes were spherical-oval, with dimensions of 2–6 x 2–8 µm (Fig. 6-C). Isolate Y-9, also isolated from Balad district but from strawberry fruits, which distinguished by its pale pink colonies, a gelatinous texture, and a round shape. Its cell shapes are oval, with dimensions of 3-4 x 5-6 µm (Fig. 6-D), finally, the isolate Y-5 isolated from Al-Alam district was cream-coloured colonies, gelatinous texture, round shape, and spherical-oval cells, with dimensions of 4-6 x 8-10 µm (Fig. 6-E)

Table 2. Macroscopic and microscopic phenotypic characteristics of yeast isolates.

Characteristics	Yeasts Isolates				
	Y-6	**Y-3**	**Y-8**	**Y-9**	**Y-5**
Colonial colour	Pink	light orange	white - creamy	pale pink	creamy
Colonial texture	gelatinous	mucous	gelatinous	gelatinous	gelatinous
Colonial shape	circular	circular	circular - irregular	circular	circular
shape of yeast cells	Spherical - oval	Oval - elliptic	Spherical - oval	oval	Spherical - oval
Cell dimensions (µm)	3-2× 5-6	1.5–4×4–12	2–6×2–8	3-4×5-6	4-6× 8-10

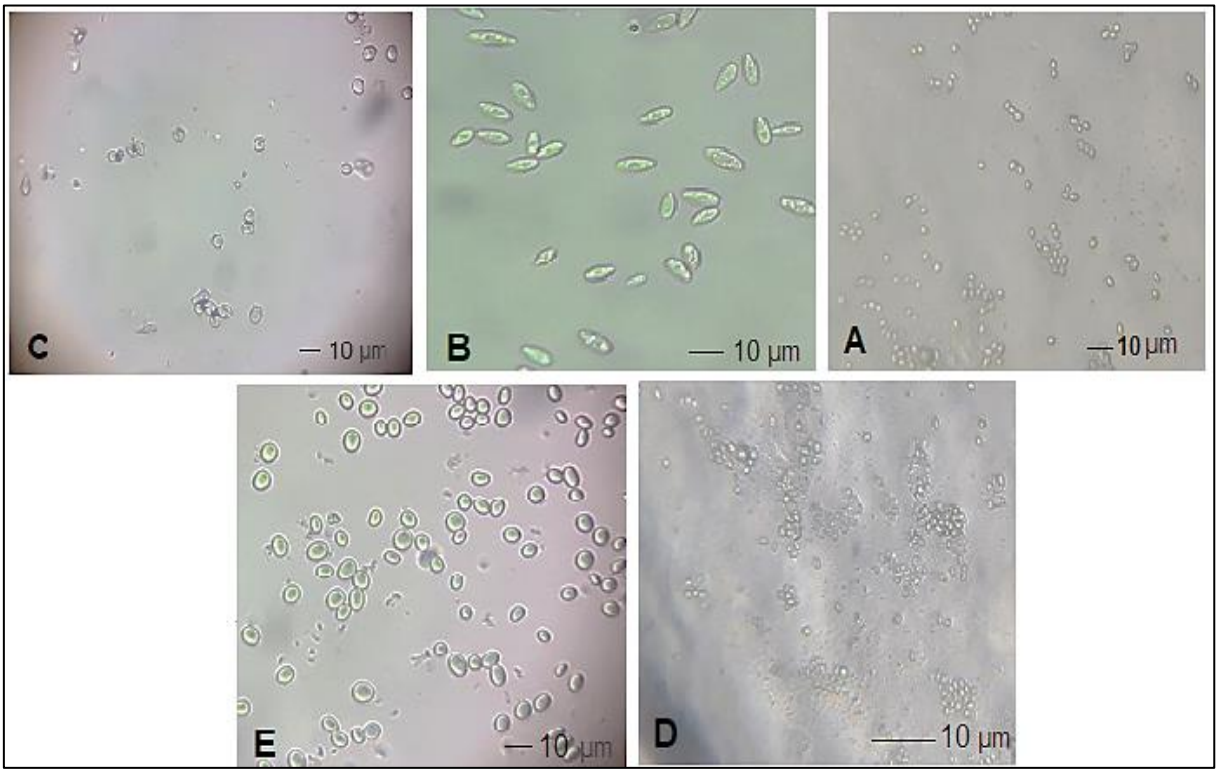

Figure 6. Some macroscopic and microscopic phenotypic characteristics of the yeast isolates.

3.7. Enzymes Activity of the Highly Antagonistic Yeasts

3.7.1. Chitinase Activity

Figure (7) showed that all the selected yeasts had the chitinase activities, it is noted that the isolate Y-9 achieved the highest activity of the chitinase enzyme, reaching 3.44 units/ml compared to other isolates, followed by isolate Y-5 with a chitinase activity of 3.12 units/ml, while the isolate Y-8 achieved the lowest activity of chitinase, reaching to 2.43 units/ml.

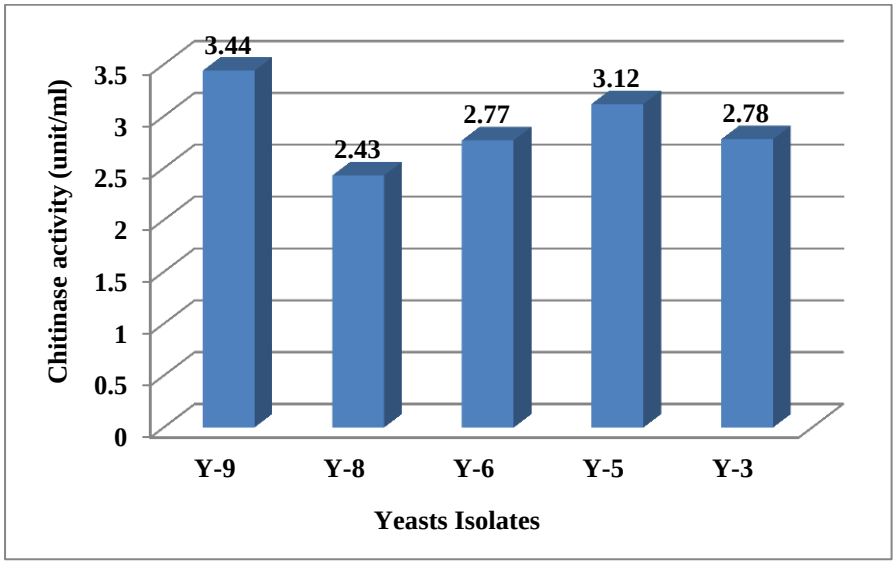

Figure 7. Chitinase activity of the highly antagonistic yeasts (LSD $_{0.05}$; 0.43).

3.7.2. β-glucanase Activity

The results of figure (8) showed that all the selected yeast isolates were producing β-glucanase, the isolate Y-9 achieved the highest β-glucanase, which was 1.82 units. / ml, followed by the Y-5 and Y-3 isolates with enzymatic activity of 1.67 and 1.51 units/ml, respectively, while the lowest β-glucanase activities were in the filtrate of Y-8 and Y-6 isolates, which reached to 1.27 and 1.22 units. /ml respectively.

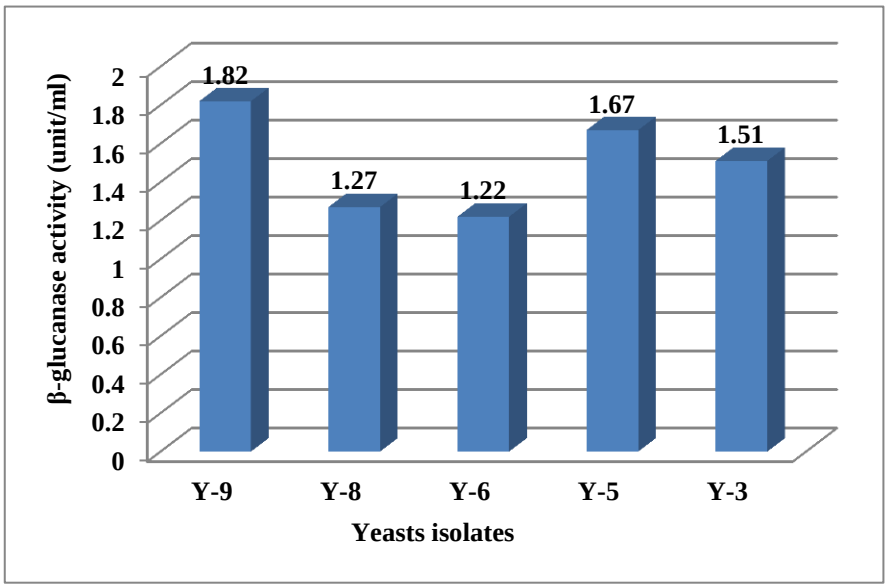

Figure 8. β-glucanase activity of the highly antagonistic yeasts (LSD 0.05 ; 0.21).

3.8. Molecular Diagnosis of Yeast Isolates

The yeast isolates were molecularly identified based on the analysis of the nucleotide sequences of the gene (5.8S rRNA), as the sequences of the nitrogenous bases of the product of the polymerase chain reaction of the gene (5.8S rRNA) were determined. Sequences obtained from the Korean company, Bioneer, were analyzed using the National Center for Biotechnology Information (NCBI) website within the sub-window Blast, then the secondary sub-window was chosen as Nucleotide blast. Figure (A1) showed the bands of genomic DNA resulting from electrophoresis. Figure (B1) showed the electrophoresis of the PCR product using the fungal universal primers. The results showed the

presence of bands with a size of 600 base pairs, which indicates the return of the PCR product to the yeasts. Table (3) showed that the nucleotide sequences of the 5.8S rRNA gene of the yeasts isolated in this study matched 99.19-99.51% with yeasts registered in the World Heritage Bank. The yeast isolates coded Y-5, Y-9, Y-8, Y-3 and Y-6 were molecularly identified as *S. cerevisiae, Rhodotorula glutinis, Zygosaccharomyces rouxii, Rhodotorula mucilaginosa and Rhodotorula glutinis*, respectively.

Yeast species were recorded for the first time as Iraqi isolates in the world Gene Bank; *R. glutinis* isolate Has.AA-44, *R. glutinis* isolate Has.AA-47, *R. mucilaginosa* isolate Has.AA-45 and *S. cerevisiae* isolate Has.AA-46. and *Z. rouxii* isolate Has.AA-48 under the accession numbers OQ108347.1, OQ108351.1, OQ108369.1, OQ108376.1, and OQ108370.1, respectively.

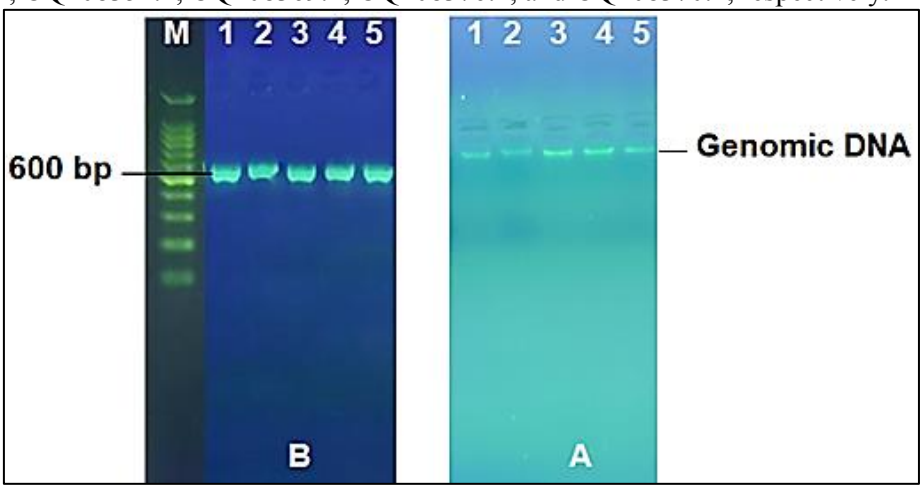

Figure 9. (A) Gel electrophoresis of genomic DNA extraction from the samples , 1% agarose gel at 5 vol /cm for 1hour. (B); PCR product the band size 600 bp. The product was electrophoresis on 2% agarose at 5 volt/cm^2. 1x TBE buffer for 1 hour.(1=Y-3, 2= Y-5, 3= Y-6, 4= Y-8, 5= Y-9).

Table 3. Molecular identification of yeasts isolates according to the identical sequences of the ITS region within 5.8S rRNA gene with the yeasts strains in the NCBI.

The most compatible yeasts species	Accession number	Country	Similarity (%)	yeasts strains recorded in the World Genetic Bank	Accession number of yeasts registered in this study
Rhodotorula glutinis	MT635318.1	Poland	99.26	*Rhodotorula glutinis* isolate Has.AA-44	OQ108347.1
Rhodotorula glutinis isolate SZ-2 10-3II3	MK123423.1	China	99.19	*Rhodotorula glutinis* isolate Has.AA-47	OQ108351.1
Rhodotorula mucilaginosa NUBS21009	LC628682.2	Japan	99.46	*Rhodotorula mucilaginosa* isolate Has.AA-45	OQ108369.1
Saccharomyces cerevisiae isolate 10-1359	MF375634.1	Hungary	98.94	*Saccharomyces cerevisiae* isolate Has.AA-46	OQ108376.1
Zygosaccharomyces rouxii strain C3-1	MH669504.1	China	99.51	*Zygosaccharomyces rouxii* isolate Has.AA-48	OQ108370.1

4. Discussion

The results showed that there is a diversity of yeasts isolated from strawberries according to the plant part and geographical area, the reason for this is attributed to the adaptation of these yeasts in certain

regions and not others as a result of the appropriateness of the environments of those regions such as the climate , as well as the contents of the elements and nutrients in the soil, which encourages its settlement, growth and reproduction, as well as its ability to penetrate and colonize plant tissues of the host and compete with other organisms [23,24,25]. The plant host and its various parts such as roots, stems, leaves, and fruits are considered important factors that encourage the growth of yeasts. These factors may be determined the types of yeasts. Other reason may be attributed to the metabolic secretions produced by these parts, and this fact is consistent with the study of [26,19] when isolating specific types of microorganisms, including fungi and bacteria, from several plant families. The diversity of yeast isolates was reflected in their effect on the pathogenic fungus *B. cinerea*. The results showed that 10 isolates out of a total of 16 isolates showed an inhibitory effect on the growth of the pathogenic fungus. Also, the inhibitory effect of the inhibitory isolates differed in the condition of yeast cells and their filtrates. This may be due to the quantity and quality of metabolites secreted by yeasts in the liquid medium, which are in a higher quantity compared to cell suspension, while the effect of cell suspension is due to their spatial and nutritional competition with the pathogen [27]. As well as the activity of iron chelation and excretion of secondary metabolites [13]. Some important metabolic secretions produced by yeasts that have anti-fungal activity such as 3-amino-5-methylhexanoic acid, biphenyl-2,3-diol and sinapaldehyde are secreted by *M. pulcherrima* yeast cells, as they showed high inhibitory activity in post harvesting conditions against *B. cinerea* infection. [28]. The lack of ability of uninhibited yeasts to the pathogenic fungus can be explained by their weak growth and weak metabolic activity, and this is consistent with the study of [17] when testing a number of actinomycetes against the fungus *B. cinerea*.

The current study also proved that the enzymatic activity such as chitinase and glucanase is one of the most important active substances that have an inhibitory role in the growth of the pathogenic fungus *B. cinerea*. These enzymes decompose chitin and glucan, which are the two main components of the hyphae wall of the pathogenic fungus, and represent the first line of protection for it, by these enzymes activities, leads to weakening of the hyphae and cell death, these results are in line with a number of previous studies [19,20]. According to the current study, the highest inhibition of the pathogenic fungus was recorded by the isolates that gave the highest activity of the chitinase and glaucinase. It is noted from the results that there is a discrepancy in the production of chitinase and glucanase as a result of the genetic structure of yeasts, as they vary genetically and thus are reflected in their physiological and phenotypical characteristics, in addition to the importance of this enzyme for the selected yeast isolates for its role in the growth and vitality of yeasts, as it participates in the analysis of chitin into small and simple particles of N -acetyl glucoseamine provides yeasts with amino and glycemic compounds [29]. In order to select yeast isolates within the biological control program and the integrated management program, the effect of cell suspension of yeast isolates on infecting strawberry fruits was studied. The results showed that isolates Y-2, Y-10, Y-13, Y-15, and Y-16, which inhibited the pathogenic fungus, led to the appearance of color changes and tissue decomposition in strawberry fruits, while isolates Y-3, Y-5, Y-6, Y-8, and Y-9 did not show any negative effect on strawberry fruits, although they were inhibitory to the pathogenic fungus *B. cinerea*. Therefore, the last isolates are considered promising isolates that can be used as biological control agents against this pathogen. The possible reasons that can explain these results are the fact that the isolates of yeasts affecting strawberry fruits have enzymatic activity such as pectinase, cellulase, and other enzymes that are higher than non-influencing yeasts, as these enzymes are considered hydrolysis enzymes for the components of plant cell walls and contents [30]. The yeast isolates that showed the highest inhibition of pathogenic fungi that did not affect strawberry fruits were molecularly identified based on the analysis of the nucleotide sequences of the 5.8SrRNA gene. Despite the high compatibility of the isolated yeast types with the international strains, the match was not 100%. This may be due to the fact that these isolates are different genetic structures that were isolated from different niches with a reflection of the environmental impact in their genetic structure, because their environments are from different geographical regions with different rates of environmental pollution, especially spraying with different chemical pesticides, and the reason for this may be attributed to the occurrence of matings that occur in yeasts [31].

Conclusion

Among all the yeast isolates, the molecularly identified yeasts; *Rhodotorula glutinis, Rhodotorula mucilaginosa*, *Saccharomyces cerevisiae and Zygosaccharomyces rouxii* are the most efficient agents in inhibiting the pathogenic fungus *Botrytis cinerea*. in addition, these yeasts don't show any negative effect in the strawberry fruits. *Rhodotorula glutinis* isolate Has.AA-44 showed the highest activities of, chitinase and β-glucanase which predicts it as a promising biological control agent.

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First Record at Molecular Level for *Fusarium culmorum* Causing Rot Seeds on *Broad bean* Plants in Iraq

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Abstract. The seeds of Broad bean were collected from Rabia, Qubba and Fadhiliyah regions, and the fungi were isolated with different frequencies, as the fungus *Aspergillus flavus* and *Aspergillus* niger reached 37.5% and 37.5%, respectively, and the frequency of appearance of the fungus *Penicillium* spp was 8.75%. As for the two fungi, *Fusarium culmorum* and *Alternaria alternate*, their occurrence frequency was 7.5% and 2.5%, respectively. As for the fungus. *Stemphlium* sp and *Alternaria cheiranthi* had a frequency of 1.25% and 1.25%, respectively. The fungus *F. culmorum* was diagnosed based on the morphological characteristics and then the pathogenicity of the fungus was confirmed by applying Koch's hypotheses. . And by testing the molecular diagnosis and then identifying *F. culmorum* by internal copy matching (ITS) in the ribosomal region that retains the DNA, all sequences in (ITS) proved to be identical to the fungus *F. culmorum* in the database in the GenBank, with a similarity rate of 98.34%. Recording of the Iraqi isolate under accession number MT276076.1. This is the first record of the fungus *F. culmorum*, the cause of bean seed rot, in Iraq. Among the pathogenesis of the fungus in the plastic house, the infected plants showed a yellow color and wilting, and long red and brown stripes appeared on the surface of the roots. The infected plants were pale in color and stunted compared to healthy plants. The fungus also caused the death of seedlings before and after germination.

Keywords. Molecular Level, Legume crops, Faba bean plant.

1. Introduction

Legume crops are among the most important food crops belonging to the legume family *Leguminosae* Which is one of the plant families that spread widely compared to the plant families, which occupies the second place after the Poaceae family. The legume family includes about 20,000 species and700 species, and are found all over the world [1,2]. The faba bean plant, L Broad bean, is one of the plants known to ancient man. It was known to the ancient Egyptians, Romans, and Greeks since 6 thousand years BC. It is native to North Africa and southwest Asia. It is a winter crop dating back to The leguminous family, of economic importance, the seeds of the broad bean contain protein at a high rate of 25-40%, carbohydrates by 56%, as well as a quantity of fiber, mineral elements, oils, vitamins, especially vitamin B, and high levels of phytic acid. The beans also contain a group of amino acids such as leucine and Lysine, as well as arginine, as well as its seeds contain important elements needed by the human body [3,4]. Diversifying protein sources in food and increasing consumption of plant proteins can reduce health and environmental risks associated with excessive production and consumption of animal protein. in succession betweentwo crops, by improving the soil, as it fixes

atmospheric nitrogen in a symbiotic relationship with rhizobium bacteria through the formation of root nodes, because the bean crop is important and is exposed to many diseases that cause great losses to the crop, including insect pests, fungal diseases, viral diseases, snake worms and parasitic flowering plants. Root and stem rot disease, bean rust disease, and ascochyta blight are among the most important diseases that affect bean crops [5-7]. Fusarium is one of the ten most dangerous fungi that affect plant production and cause large economic losses [8,9]. The fungus infects the plant at all stages of its growth. Symptoms appear in the form of wilting, with a change in the color of the plant to yellow. The root appears with long red or brown stripes. The main root is dark brown in color, and most of the root hairs are lost and black in color, and thus affects the plant. Pale color and in severe infections affect on production, as the quantity of the product decreases, as well as the smallness of the fruits and seeds, and then the yellowing and drying of the plant [10].

2. Materials and Method

2.1. Sample Collection and Isolation of Fungi

Seeds were collected from the local markets, which were represented by (bread peas from the regions of Rabia, Al-Qubba and Al-Fadhiliyah) at a rate of 1 kg for each type of these areas, and were placed in polyethylene bags. The samples were transferred to the laboratory in the Plant Protection Department in the College of Agriculture and Forestry / University of Mosul for the purpose of isolating the fungi associated with the seeds. The seeds were washed with tap water to get rid of dust and residues, then they were sterilized in a 1% sodium hypochlorite solution for three minutes, then washed with sterile distilled water to remove the residuals of the sterilized material. Then, the seeds were transferred using sterilized alcohol and heat flame tweezers to the nutrient medium.Potato Dextrose Ager (PDA), which was prepared by adding 39.5 g/L of distilled water, was shaken until the nutrient medium was mixed, and sterilized with an Autoclave device to which the antibiotic Streptomycin was added at a concentration of 100 mg/L. After sterilization, the dishes were incubated in the incubator at a temperature of 25 C for (5-7) days with daily monitoring during the incubation period ±°.

2.2. Identification of Fungi Isolated from Seeds

The taxonomic keys developed by [11-14], and the different characteristics of the spores, their arrangement, sporophyte, and mycelium of the fungus were relied upon, and the diagnosis was relied upon by taking part of the edge of a newly developed colony placed on a glass slide (slide) for microscopic examination, after which it was inoculated on the slant habitats from PDA medium And kept in the refrigerator at a temperature of 4° salts for use.

2.3. Calculating the Percentage of Fungus Appearance

The percentage of fungal emergence was calculated as follows:

$$\text{Fungal frequency (\%)} = \frac{\text{No. of pieces showed fungus}}{\text{Total No. of pieces in the sample}}$$

2.4. Molecular Diagnosis of F.culmorum

Molecular diagnostic technology is based on the extraction of deoxyribonucleic acid DNA from the pathogen colony that is purified by the isolation singie spore method and diagnosed initially, the DNA was extracted using a DNA extraction kit (prepared for this purpose and available in the markets and follows the working method described by the manufacturer attached to the extraction section). Molecular diagnosis was done by using polymerase chain reaction (PCR) technology and (primers) designed for diagnosis, as they relied on sources and research.

2.5. Extraction and Amplification of Genomic DNA

Fungi developed *F. culmorum* At a temperature of 27 °C on a food medium° Potato Sucrose Booth (PSB) for 10 days, then the mycelium was obtained by filtration with Whitman1 filter paper where

DNA was extracted from the fungi using TMDNA Miniprep Guide D6005, according to the company's protocol (ZYMO, USA)

2.6. Electrophoresis Technology for an Extract DNA

Electrophoresis was performed to determine cut off DNA after extraction or to detect the PCR result while DNA is present to discriminate the bundle size of the PCR result on an agarose gel.

2.7. Preparation of the Acarose Gel

The agarose gel preparation method was adopted according to the previously used method Sambrook et al.,[15].

2.8. Loading and Transporting

Put in the first hole a molecular weight indicatorDNA Marker by mixing 3 µL of the molecular weight indicator with the loading dye. In the last hole, 5 µL of the extracted DNA was placed in it and then exposed to an electric current of 7 v/c2 for 1-2 hours until the dye reached the other side of the gel. After the migration is completed, the gel is examined with a UV device at a wavelength of 336 nm, after placing the gel in a bath containing 3 µl of safe DNA dyeing solution and 500 ml of distilled water, to know the quality of the bands in the gel.

2.9. Gene Detection ITS using Polymerase Chain Reaction

Gene detection was performedInternal Transcribe Spacer (ITS) Using the two primers for implication, a part of the ITS was amplified using the forward and reverse primers shown in Table (1) produced by IDT (Integrated DNA Technologies, Canada).

Table 1. The nucleotide sequence used in the diagnostic process.

Primer	Sequence	Tm °C	GC %	Product size
Forward	TCCGTAGGTGAACCTGCGG -3′-5′	60.3	50%	650-500
Reverse	TCCTCCGCTTATTGATATGC-3′-5′	57.8	41%	base pair

Amplification was performedPCR volume of 25 µL containing 1.5 µL DNA, each of primer and reverse primer and 5 µL (poml 10) and 5 µL Taq PCR PreMix (Intron, Korea) then distilled water was added to a 25 µL tube and the device was run according to the program in table (2).

Table 2. Program for the interaction of primers with the genome of fungal isolates in the polymerase chain reaction PCR.

The number of courses	Time (minutes)	m heat	Phase	Number
1	3:00	95	Primary danderPrimary Denaturizing	1
	45:00	95	danderDenaturizing	2
35	1:00	52	coloringAnnealing	3
	1:00	72	initial elongationElongation	4
1	7:00	72	final elongationFinal Elongation	5

Table 3. The Components of the Maxime PCR PreMix kit (i-Taq).

Material	Volume
i-Taq DNA Polymerase	5U/µl
DNTPs	2.5mM
Reaction buffer (10X)	1X
Gel loading buffer	1X

2.10. Electrical Migration of a Product PCR

The electrophoresis technique was used on the acarose gel, as a molecular weight indicator was placed in the first holeDNA Marker by mixing 3 µL of the indicator MW with the loading dye, and in the last hole, 5 µL of PCR product was placed in it, and then exposed to an electric current of 7 v/c2 for 1-2

hours until the dye reached the other side of the gel. After the migration is completed, the gel is examined with a UV device at a wavelength of 336 nm, after placing the gel in a bath containing 3 µl of safe DNA dyeing solution and 500 ml of distilled water, to know the quality of the bands in the gel.

2.11. Pathogenicity of F.culmurum in the Green House

This experiment was carried out in the plastic house of the Plant Protection Department in the College of Agriculture and Ghalibat at the University of Mosul, where the soil was sterilized using a compound (OXY) and the soil was placed in pots of (5) kg capacity in which the seeds were sown using the isolated fungus suspension F. culmorum for legumes (the seeds were soaked separately in the fungus suspension for an hour) with five seeds per pot and with three replications. As for the comparison treatment, the seeds were soaked with distilled water After that, the plants were left until the disease symptoms appeared. After the appearance of disease symptoms on peas, chickpeas, and broad beans, these infected plants were re-isolated to confirm the pathogencity of the pathogenic fungi used in the study and to apply Koch's patulates' hypotheses.

3. Results and Discussion

3.1. Recurrence Rate and Isolation of Fungi from Broad Bean Seeds

The results showed that the studied bean seeds were contaminated with many fungi, and the frequency of appearance of the contaminated fungi differed in the seed samples, as 63 isolates were isolated from the fungi present in the bean seeds belonging to seven fungi. The results are shown in the table (4). Most of the isolates obtained from bean seeds had the highest percentage of fungus A*spergillus flavus* and *Aspergillus niger,* as the recurrence rate was 37.5% and 37.5%, respectively, followed by the *Penicillium fungus.*spp The recurrence rate was 8.75%, followed by *Fusarium culmorum* and *Alternaria alternate* had a frequency of 7.5% and 2.5%, respectively, and the lowest frequency was for .*Stemphlium* sp *and Alternaria cheiranthi,* which were 1.25% and 1.25%, respectively. And he mentioned [16]. The most common fungal genera on nine species of seeds are *A spergillus, Penicillium, Alternaria, and Fusarium.*

Table 4. Replication rate and isolation of fungi from *Vicia faba* seed*s*.

Recurrence %	Fungi
37.5	*Aspergillus flavus*
37.5	*Aspergillus niger*
8.75	*Penicillium*spp
7.5	*Fusarium culmorum*
2.5	*Alternaria alternata*
1.25	*Stemphlium.sp*
1.25	*Alternaria cheiranthi*

3.2. Isolation and Identification of Fungal Pathogens from Bean Seeds

3.2.1. Morphological Diagnosis

The results of isolation and identification of the seeds of the fungus bean plant were shown *Fusarium culmorum* It was isolated from the bean seeds, and the fungal colony was orange in color. The fungus is characterized by its abundant growth on the medium PDA It has large spores on the fungal cushion in a central manner with a diameter of 1-2 cm. The color of the fungal cushion is orange to light orange, which soon becomes brown with the age of the culture. The wall of the large spore is thick and strong, and its central region appears to be the largest in diameter, and it is curved from the dorsal side. Whereas from the ventral side, it is mostly straight, and the large conidia contain 3-4 septa, while the small conidia are non-existent, while the chlamydial spores are rapidly formed and produced in abundant numbers, and their formation may be absent in rare cases [14], Figure (1).

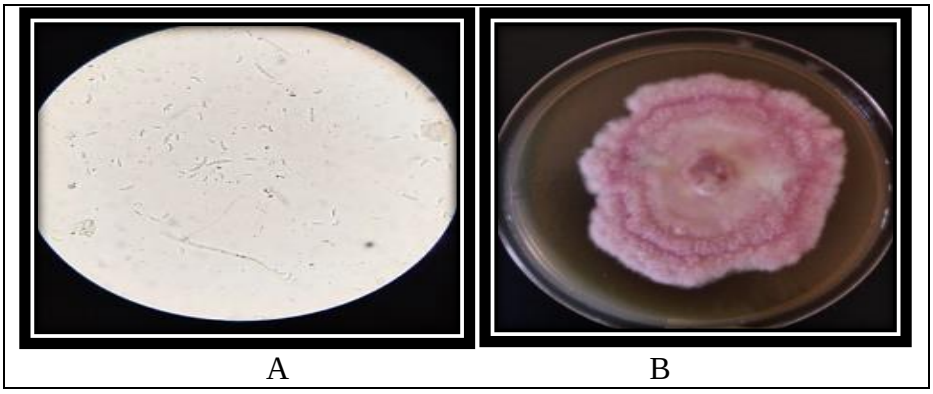

Figure 1. A - a fungus colony *F.culmorum,* B- fungal conidia F.*culmorum.*

3.3. Molecular Diagnosis

3.3.1. Extraction of Deoxyribonucleic Acid (DNA)

The results of the examination were shown by a deviceSpectrophotometer DNA density isolated from faba bean seeds infected with the fungus F. culmorum, as the isolation produced a DNA concentration of 77 ng / ml, according to the reading of the Nanodrop device.the Table (5). With a purity ratio of (1.9), this ratio is one of the indicators of the purity of the DNA, as well as extraction efficiency, and high-purity DNA is considered when the value is between 1.8-2.1, and then the use of pure and diluted DNA later in subsequent PCR reactions.

Table 5. DNA sample concentration according to Nano drop results.

Sample DNA	Nucleic acid conc.(ng Ml-1	260/280 purity
1	77	1.9

3.3.2. Polymerase Chain Reaction (PCR)

The results of the polymerase chain reaction are shown Polymerase Chain Reaction (PCR) for a pair of general primers to detect the gene in the ITS region (ITS1 and ITS4 (Table 6), which is one of the most suitable regions for diagnosis at the genus level, as well as being preserved as a result of developmental restrictions, as it distinguishes between genera and its species Figure (2). It matches what he mentioned [17].

Table 6. Genetic primers used for the detection of *F.culmorum.*

Fungi	Score	Expect	identities	Gaps	Strand
F. culmorum	782 bits (423)	0.0	440/447 (98%)	5/447 (1%)	Plus/Plus

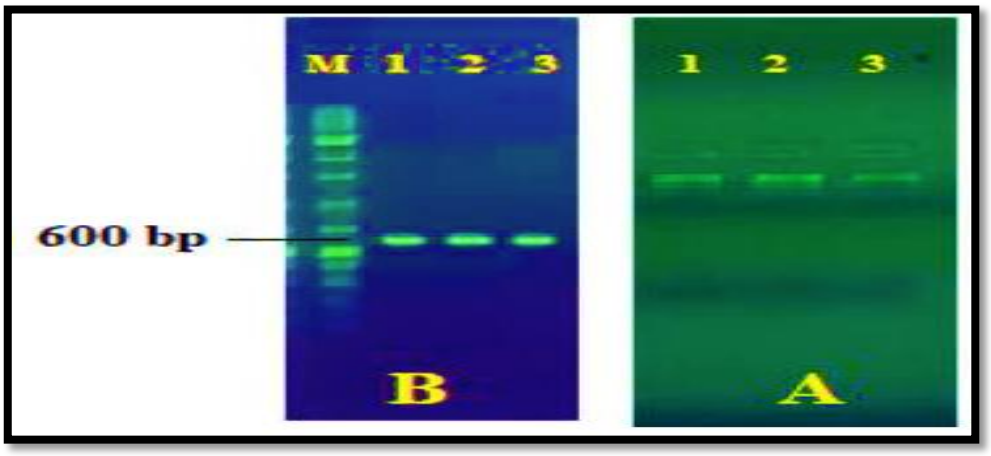

Figure 2. The beam resulting from the reaction DNA with genetic primers.

3.3.3. Nucleotide Sequence Analysis Sequencing Analysis

Afte r Perform nucleotide sequence analysis of the ITS regionAs he took afor *F. culmorum* accession number MT276076.1 compared with recorded data for the same fungi found in the NCBI databaseIt is represented in Table (7) and Figure (3).

Table 7. The nucleotide sequence used in the diagnostic process.

Primer	Sequence	Tm ^{o}C	GC %	Product size
Forward	TCCGTAGGTGAACCTGCGG -3′-5′	60.3	50%	650-500
Reverse	TCCTCCGCTTATTGATATGC-3′-5′	57.8	41%	base pair

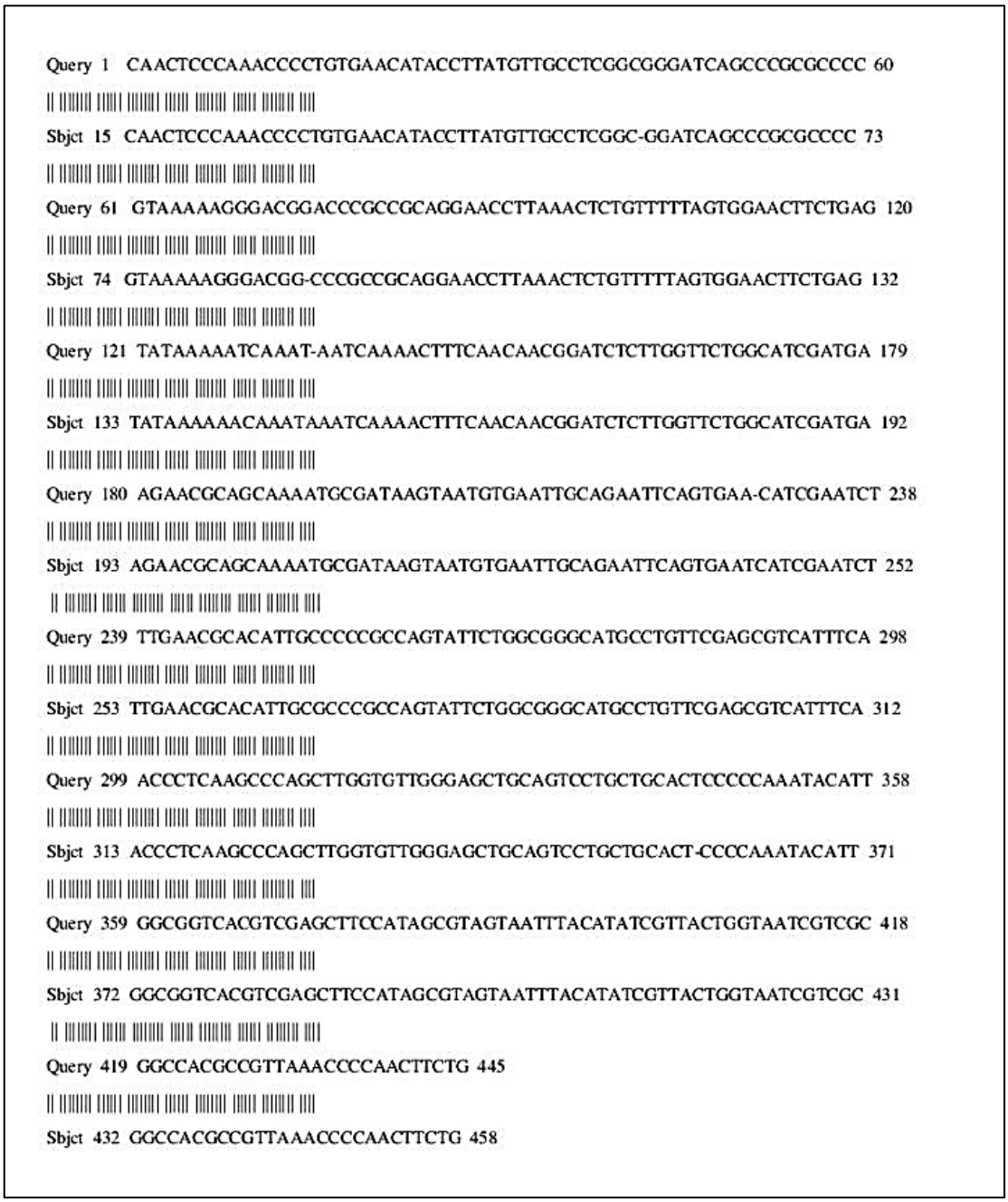

Figure 3. The sequence of nicotides for the internal reproduction regions ITSand compared with the isolation of the fungus F*usarium culmorum*With accession numberOP592243.1in the gene bank NCBI.

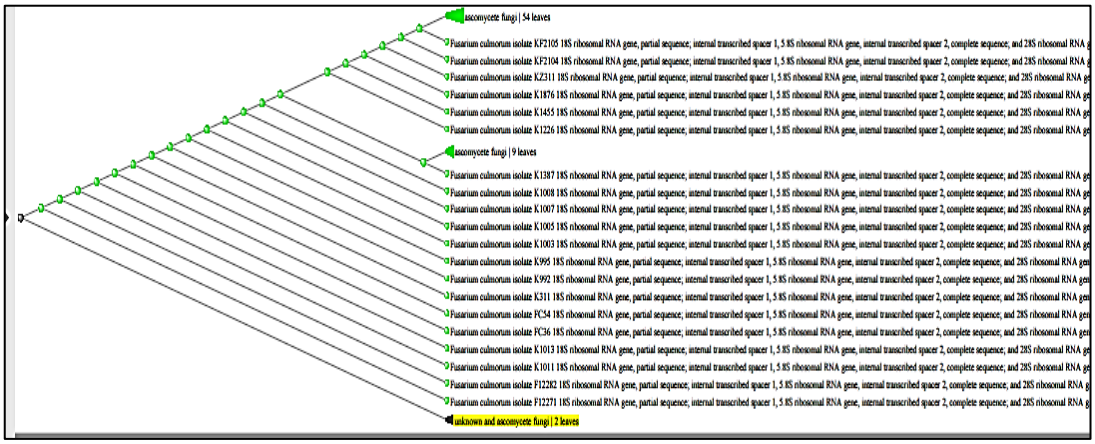

Figure 4. Genetic tree by joining the neighborneighbor-joiniog showing the phylogenetics of F.culmorum compared to the isolates recorded in Genbank. Registering fungal isolates in the gene bank of the National Center for Biotechnology InformationNational Center For Biotechnology Information (NCBI).

The results showed that the percentage of congruence withslip ato *F. culmorum* 98.43%And registered under the accession number MT276076.1.It is also noted from the genetic tree that the match rate between the isolate of F.culmorum recorded in the database and its affinity was 98.43% for the fungus F.culmorum and the closest it was. With the Polish isolation, Aliyah these results agreed with what he stated [18], on the possibility of identifying the pathogen based on a short sequence on the DNA strand within the nucleotide sequencing technique (Figure 4).

Fungus isolates were recorded *F . Culmorum* Under accession number OP592243.1 ID: in the GenBank of the National Center for Biotechnology Information (NCBI) after nucleotide sequence analysis of the ITS regionthe Fig.(5).

Fusarium culmorum strain NA1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

GenBank: OP592243.1

FASTA Graphics

Go to:

```
LOCUS       OP592243                445 bp    DNA     linear   PLN 12-OCT-2022
DEFINITION  Fusarium culmorum strain NA1 internal transcribed spacer 1, partial
            sequence; 5.8S ribosomal RNA gene, complete sequence; and internal
            transcribed spacer 2, partial sequence.
ACCESSION   OP592243
VERSION     OP592243.1
KEYWORDS    .
SOURCE      Fusarium culmorum
  ORGANISM  Fusarium culmorum
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; Nectriaceae;
            Fusarium.
REFERENCE   1  (bases 1 to 445)
  AUTHORS   Asker,N.F. and Mohamed,A.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (06-OCT-2022) Department of Plant Protect, 11.##work
            address#University of Mosul, College of Agriculture and Forestry,
            aljameaa street, mosul 41001, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
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                     /mol_type="genomic DNA"
                     /strain="NA1"
                     /isolation_source="Infected bean seeds"
                     /host="bean seed"
                     /db_xref="taxon:5516"
                     /country="Iraq"
                     /collection_date="20-Jan-2022"
                     /collected_by="Naif fahad"
```

Figure 5. Recording of mushroom isolation *F. culmorum*in the International Genebank (NCBI).

3.4. Pathogenicity of a Fungus Fusarium Culmorum on Broad Bean Sprouts in the Greenhouse

The pathogenicity of the fungus was tested *F. culmorum* isolated from the seeds of the bean plant, and the infected plants appear as shown in the figure (6) with a yellow color, withering, and long red or brown lines appear on the surface of the roots, and the plants appear in a pale color.longer sex *F usarium* spp.One of the most important causes Plant diseases that are transmitted through soil and seeds and represent a major threat to legume and grain crops [17,19]. *Fusarium* spp. Loss of germination ability, low emergence of seedlings, vascular wilt and root rot may be a loss in yield of up to 100% in sensitive varieties [19,20]. *F. culmorum* was mentioned as one of the most important pathogens that affect the bean crop and cause great damage to the crop [21,22]. He also indicated [23], that the fungus *F. culmorum* infects a number of plants, including wheat, barley, corn, oats, peas, peas, leeks, strawberries and potatoes. the fig. (6) healthy flea plant and an infected plant.

Figure 6. Infected plant and healthy plant.

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The Effect of an Aqueous Extract of Cress Seeds, Lepidium Sativum, on some Functional and Histological Parameters of the Kidneys in Tetragonal Female and Male Rats with Renal Failure Induced by Carbon Tetrachloride

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Abstract. The study was conducted on 24 female and male Sprague Dawley rats weighing 200-225 grams who were eight weeks old. They were randomly distributed into three groups of females and three groups of males: the control group, G1, and the group with renal failure caused by carbon tetrachloride (CCl4), which were injected at a dose of 0.1. ml / 100 gm of animal weight / twice a week for four weeks through the proteolytic membrane and left without treatment. The positive control sample, G2, and the third group were treated with water extract of cress seeds, Lepidium sativum. At a concentration of 10% with carbon tetrachloride (ccl4), the results of injecting ccl4 through the proteome membrane showed a significant increase ($p < 0.05$) in the levels of urea, uric acid, creatinine, and glomerular filtrate. And a significant decrease ($P<0.05$) in total protein, albumin and globulin values compared to the control or standard group. Oral administration of cress seed extract improved urea, uric acid, creatinine, total protein, albumin, and globulin levels. Cress seeds have protective effects against the effects of carbon tetrachloride poisoning on kidney function. They improve the levels of electrolytes in the blood and return them to their normal levels. Cress of potassium concentration was closer to normal, as it was recorded as 5.3 in male rats and 4.6 in female rats treated with an aqueous extract of cress seeds, compared to the group of standard animals G1. As for chloride, it showed a significant decrease $P<0.05$ in the group of untreated infected animals G2, reaching 87 in males. The rats were not affected significantly in the female group, as it scored 94 compared to the standard animal group G2, while in the group of infected animals treated with an aqueous extract of cress seeds G3, where the value of chloride in male rats was 99, while its value in females was 98 compared to the standard animal group G1. . Also, kidney tissues improved significantly after a dose of cress seeds extract, compared to the group infected with ccl4 poisoning.

Keywords. Lepidium sativum, CCl4, Kidney function, Uric acid.

1. Introduction

The renal system is exposed to several disorders. Renal insufficiency is considered one of the most severe disorders because the kidney's inability to get rid of sufficient amounts of urine will accumulate waste from metabolic processes and excess substances of organic salts and water in the body. Kidney diseases are called silent diseases because they often give warning signs, as a person can lose 90% of kidney function before he feels symptoms of the disease. Kidney disease also increases the risk of cardiovascular disease. While these problems may occur slowly and without symptoms, they can lead to kidney failure [1]. We may be exposed to many harmful chemicals that cause many diseases in our daily lives. One of them is carbon tetrachloride, an organic compound mainly exposed to breathing polluted air, water, or soil. Exposure to it in large quantities causes considerable damage and diseases to the liver and kidneys [2]. Medicinal plants play a fundamental role in the development of human culture. As a source of medicine, medicinal plants have always been at the forefront of almost all civilizations' cultures. Medicinal plants are rich resources for traditional medicines; many modern medicines are produced from these plants. The secondary metabolites produced by plants are responsible for the biological characteristics of plant species used worldwide [3]. Medicinal plants benefit health because they contain active compounds responsible for their therapeutic properties. The active compounds of medicinal plants have diverse and significant biological effects. These active substances help protect vital organs such as the liver, kidneys, heart, pancreas, lungs, brain and digestive system. It also regulates sugar, salts, and fats in the blood, activating and stimulating natural immunity. It plays a positive role in improving mental health and many other positive health characteristics [4].

2. Materials and Methods

The study was conducted in the animal house of the College of Veterinary Medicine, Tikrit University, for the period from 1/4/2022 to 30/4/2022, and it was carried out through the following:

2.1. Sample Collection and Preparation

Cress seed samples were purchased from the local markets of Salah al-Din Governorate, Tikrit, and they were diagnosed by specialized professors in the College of Agriculture - Department of Horticulture. The samples were dry and ground by an electric mixer of Chinese origin to obtain a fine powder.

2.2. Prepare the Extract

Ten grams of dried and ground cress seed powder was added to 100 millilitres of hot distilled water and left for 10 minutes. The solution was filtered using medical gauze, after which the suspension was deposited using a centrifuge at 5000 revolutions for 10 minutes twice in a row. The supernatant liquid was collected, and the water evaporated from the extract. At room temperature, dry it [5], collect it in tightly closed bottles and keep it in the refrigerator until use, and prepare the weights required in the study.

2.3. Animals used in the Study

The animals were obtained from the College of Veterinary Medicine, University of Tikrit's animal house at the age of 8 weeks, weighing 200-225 gm, Albino breed. They were placed in special cages with dimensions (19 x 25 x 21 cm). The animals were exposed to good laboratory conditions of a light cycle divided into 12 hours of light and 12 hours of darkness. The temperature is set at 24±2°C. The NRC recommended nutrition, and water was standard throughout the study period.

2.4. Design Experience

Twenty-four experimental animals, 12 males and 12 females were randomly divided into three groups, with four animals for each male and female.

- The first group (G1) was the control group (control group) without injection or dose and was given only a physiological solution.

- The second group (G2): was injected with carbon tetrachloride 0.1 / 100 g of body weight twice a week for four weeks [6].
- The third group (G3) animals were injected with CCL4 at a concentration of 0.1/100 g of body weight with the aqueous extract of cress seeds at a concentration of 300 mg/kg of body weight per day for four weeks [7].

2.5. Collect Blood Samples

After the end of the experiment, the animals were starved for 10 hours, then weighed and anaesthetized with chloroform. Then, blood samples were collected by drawing blood directly from the heart. Tubes free of anticoagulants are tested. They were left for about a quarter of an hour in a water bath at 37 ° C until coagulation, then placed in a centrifuge for 15 minutes at 3000 rpm, and the serum was withdrawn. By a miniature pipette and placed in new, cleaned plastic tubes (flat tubes) and kept at -20 °C until special biochemical tests are performed that include Urea, Uric Acid, Creatinine, Total Protein, Albumin, and using many standard solutions (Kits) of French origin. Serum globulins were estimated according to the following equation Globulin concentration (g/dl) = Total Protein Conc. - Albumin Conc. To measure the glomerular filtration rate (GFR), the following equation was used according to the method. Cockcroft-Gault formula [8].

2.6. Histological Study

The kidneys were excised from the animal and kept in a 10% formalin-butyricane solution. It was extracted after 48 hours from formalin and washed several times with ethyl alcohol at a concentration of 70%. Then she underwent a series of operations based on the method used by [9].

2.7. Statistical Analysis

The results were analyzed statistically using [10], according to a one-way analysis of variance. The mean of the coefficients was tested using Duncan's multiple range test at a significant level ($P<0.05$) to determine the significant differences between the groups.

3. Results and Discussion

The results of table (1) show the concentration of urea in the blood serum of female and male rats with renal failure induced by carbon tetrachloride compound CCL4 after it was injected through the proteolytic membrane. Compared to the standard sample G1 39 and 37, respectively, and showed significant differences at $P<0.05$ between the male and female groups. The reason may be due to the difference in male metabolism compared to female metabolism and the difference in sex hormones between the two groups. The value of urea decreased and was recorded at 46 compared to the infected group without G2 treatment. In contrast, the group of animals treated with an aqueous extract of cress seeds did not show any significant differences compared to the control group, as it recorded a value of 37. While the values of creatinine showed a significant increase of $P>0.05$ in the group of animals with renal failure treated with carbon tetrachloride CCL4 and without G2 treatment, as the value of creatinine in male rats was 0.80. In female rats, it was 0.90 compared to the standard group G1, as it was 0.19 and 0.20 in male and female rats. Respectively, the group of animals treated with an aqueous extract of cress seeds recorded a significant decrease, $P<0.05$, as the creatinine values in male and female rats were 0.29 and 0.26, respectively, compared to the group of infected animals without treatment. G2

The uric acid values showed a significant increase, $P>0.05$, in the group of infected animals without treatment G2 compared to the standard group G1. The values were 2.4 and 2.2 in male and female rats, respectively. Compared between males and females, the values of uric acid LAD did not show significant differences between males and females. The animals were recorded infected and treated with an aqueous extract of cress seeds, with values of 2.5 and 1.5 in males and females, respectively.

Table 1. Urea, creatinine, uric acid and glomerular filtration rate in blood.

Adjective/ transactions	Urea		Creatinine		Uric lion		Glomerular filtration	
	Males	Females	Males	Females	Males	Females	Males	Females
Control sample	39 ±6.82 D a	37 ±6.11 Ea	0.19 ±0.021 B a	0.2 ±0.071 B a	1.8 ±0.64 C a	1.4 ±0.59 C a	2.31 ±1.06 A a	1.95 ±0.89 A a
Infected without treatment	63 ±11.2 A b	86 ±9.47 A a	0.80 ±0.14 A a	0.9 ±0.27 A a	2.4 ±1.30 B a	2.2 ±0.76 B a	0.50 ±0.06 D a	0.44 ±0.06 D a
Infected treatment extract Cress love	46 ±8.88 C a	37 ±4.28 E b	0.29 ±0.051 B a	0.26 ±0.08 B a	2.5 ±1.22 B a	1.5 ±0.88 C a	1.49 ±0.44 BC a	1.45 ±0.82 B b

As for glomerular filtration, the group of animals with renal failure and treated with carbon tetrachloride (CCL4) recorded a significant decrease (P<0.05) in the glomerular filtration of the kidneys of the group of infected animals without treatment, as it recorded values of 0.50 and 0.44 in male and female rats, respectively, compared to the standard group G1, which recorded filtration values. The glomeruli were 2.31 and 1.95 in male and female rats, respectively. At the same time, the aqueous extract of cress seeds improved the glomerular filtration of the group of animals with renal failure after dosing them with the extract, as the values were 1.49 and 1.45 in male and female rats, respectively, compared to G2.

The results of our study agreed with the findings of [11], which recorded a decrease in urea and creatinine levels in white rats induced by oxidative stress by carbon tetrachloride CCL4, which ate 5% per day of cress seed powder in the diet. Cress seeds contain effective compounds such as flavones, phenols and antioxidants that can remove free radicals from the body, especially tannin alkaloids, glycoside compounds, amino Flavonoids and amino acids such as (glutamine cysteine, and glycine) which can reduce oxidative damage to renal cells and glomeruli and increase Glomerular filtration rate and reducing problems resulting from free radicals [12], that the diuretic effect of cress seed extract, which may be one of the reasons for the decrease in urea and creatinine, is the diuretic effect of cress seeds, and the diuretic effect may be due to the containment of cress seeds on flavonoids, saponins, and organic acids [13].

The results agreed with [14], where cress supplementation with diet led to a significant decrease in uric acid, urea and creatinine in blood in rats with hyperuricemia compared to the infected group (positive).

Table 2. Blood proteins in male and female rats with renal failure.

Adjective/ transactions	Total protein		The two albums		Globulin	
	Males	Females	Males	females	Males	Females
Control sample	6.5 ±1.88 A a	6.9 ±2.08 A a	4.3 ±1.79 B a	4.4 ±1.48 A a	2.2 ±0.71 B a	2.5 ±0.99 A a
Infected without treatment	6.2 ±2.66 AB a	5.6 ±1.41 C b	4.1 ±0.97 B a	3.5 ±1.28 B b	2.1 ±0.64 B a	2.1 ±0.68 A a
Infected treatment extract Cress seeds	5.8 ±1.89 B a	6.2 ±1.84 B a	4.8 ±1.55 A a	3.6 ±1.87 B b	1.0 ±0.091 C b	2.6 ±1.08 A a

The results of Table (2) show a significant decrease, P < 0.05, in the total protein values of the group of animals with renal failure induced by carbon tetrachloride (CCL4). Animals with renal failure treated with an aqueous extract of cress seeds showed a significant decrease, P<0.05, and the total protein values were 5.8 and 6.2 compared to the control group, G1.

The results of the two albumins of the infected animal's group without G2 treatment did not show significant differences compared to the standard group G1, as it recorded 4.1 in male rats and a significant decrease P < 0.05 amounting to 3.5 compared to male rats and the standard group G1.
The albumin value recorded a significant increase, P>0.05, amounting to 4.8 in the group of males treated with water extract of cress seeds, G3, amounting to 4.3 compared to the standard group G1. The albumin value recorded a significant decrease, P>0.05, amounting to 3.6 in female rats treated with the water extract of cress seeds, compared to the standard group G1. 4.4.
While there were no significant differences between the standard group and the group of infected animals left without treatment, as the value of globulin in males was 2.2 and 2.1 and in females 2.5 and 2.1, respectively. A significant decrease of P<0.05 was recorded in the group of males treated with aqueous extract of cress seeds G3, reaching 1.0 compared to G1, while no significant differences appeared between the group of females treated with aqueous extract of cress seeds G3 compared to G1 and G2. This decrease in total protein, albumin, and globulin may be due to the toxicity of carbon tetrachloride (CCL4), which leads to a defect in protein metabolism and, thus, rapid degradation of proteins. Increased rate of free amino acids and decreased turnover of proteins. The results are consistent with the findings of [15,16], that this decrease may be due to the toxicity of NaNO2, which leads to a defect in protein metabolism and, thus, rapid degradation of proteins. The decrease may result from oxidative stress in the kidneys, characterized by the loss of kidney proteins. Protein area through urine Since proteins are essential in the internal and external metabolic processes as well as their presence within the structure of the cell membrane of the cell and are involved in the synthesis of enzymes necessary to detoxify substances entering the body; albumin may be used as an antioxidant and thus a decrease in its levels occurs [16].

Table 3. Blood electrolytes in the serum of standard, male and female laboratory rats with renal failure.

Adjective/ transactions	Calcium		Phosphorous		Sodium		Potassium		Chloride	
	Males	**Females**	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**
Control sample	12.5 ±3.34 A a	11.6 ±1.96 AB a	6.1 ±1.66 A a	5.2 ±0.64 A a	137 ±19.9 A a	142 ±7.22 A a	4.3 ±1.24 C a	4.1 ±1.33 Da	90 ±11.9 C b	94 ±8.97 BC a
Infected without treatment	11.9 ±2.88 B a	11.1 ±1.54 C a	5.7 ±2.30 BC a	5.2 ±0.84 A a	134 ±21.3 A a	137 ±6.99 A a	7.9 ±3.36 A a	7.2 ±2.11 A a	87 ±15.2 CD b	94 ±9.14 BC a
Infected treatment extract Cress seeds	11.9 ±1.69 B a	11.8 ±1.99 A a	5.3 ±1.33 C a	5.1 ±0.88 A a	137 ±22.6 A a	141 ±9.22 A a	5.3 ±2.13 B a	4.6 ±1.57 D a	99 ±14.3 B a	98 ±6.66 AB a

Table (3) shows the concentration of blood electrolytes in the serum of male and female standard G1 rats with renal failure after exposing them to carbon tetrachloride (CCL4) after injecting them through the protein membrane G2 infected and treated with aqueous extract of cress seeds G3, where a significant decrease P<0.05 is observed in each Of calcium and phosphorus, as calcium in male rats reached 11.9 for both groups G2 and G3, while it reached 11.1 and 11.8 in female rats, respectively, compared with the standard group G1. As for phosphorus, it was 5.7 and 5.2 in males and females of the group of infected animals without treatment, G2, respectively, and 5.3 and 5.1 in males and females of rats, the group of animals treated with aqueous extract of cress seeds, G3, respectively.
While sodium did not show any significant differences in males and females. As for potassium, the group of infected and untreated animals recorded a significant increase, P>0.05, reaching 7.9 in male rats and 7.2 in females, compared to standard animals G1. It was recorded as 5.3 in male rats and 4.6 in female rats treated with an aqueous extract of cress seeds, compared to the standard group of animals, G1.
As for chloride, it showed a significant decrease (P < 0.05) in the group of infected untreated animals, G2, as it reached 87 in male rats, while it was not significantly affected in the group of females, as it scored 94 compared to the group of standard animals, G2, while in the group of infected animals treated with water extract of cress seeds G3, where the value of chloride in male rats was 99, while its value in females was 98, compared with the group of standard G1 animals.

The results are convergent with [14], where the values of sodium in the positive group were 115. For animals that were given cress seeds at a concentration of 5%, it was 130, compared to the standard group, which was 137. Potassium values significantly increased, reaching 6.6 in the positive control group and 5.7 in the control group. Cress group compared to standard control group 3.8
The results agree with [13], where the percentage of chloride was 99.8 in rats exposed to sodium nitrite and treated with water extract of cress seeds.

3.1. Histological Study
Figure (1) represents the standard group of female rats. The image shows that the renal cortex is covered with a fibrous capsule of low thickness consisting of fibres of colloidal connective tissue. The parenchyma of the cortex contained lobed renal glomeruli surrounded by the renal space and Bowman's capsule and the proximal coiled tubules lined with pyramidal cells with a narrow tubular lumen. The distal convoluted tubules are lined with simple cuboidal cells and have a wide cavity. As for the standard group of males, Figure (2) shows that the renal cortex contained normal glomeruli in terms of shape and structure, surrounded by a narrow space, Bowman's capsule, and all proximal and distal coiled tubules were in regular shape, except for the presence of a cavity for a limited number of tubules.

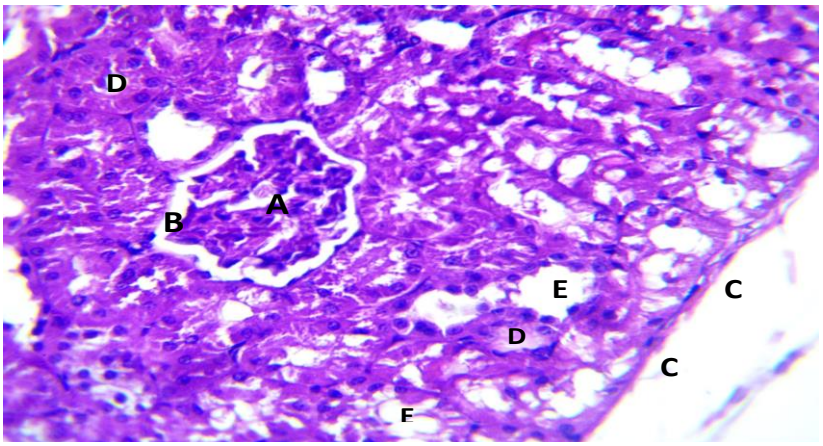

Figure 1. The standard group of females, the renal cortex, Renal glomerulus (A), the capsular space (B), the renal capsule (C), the proximal convoluted tubules (D) and the distal (E) H2EX40.

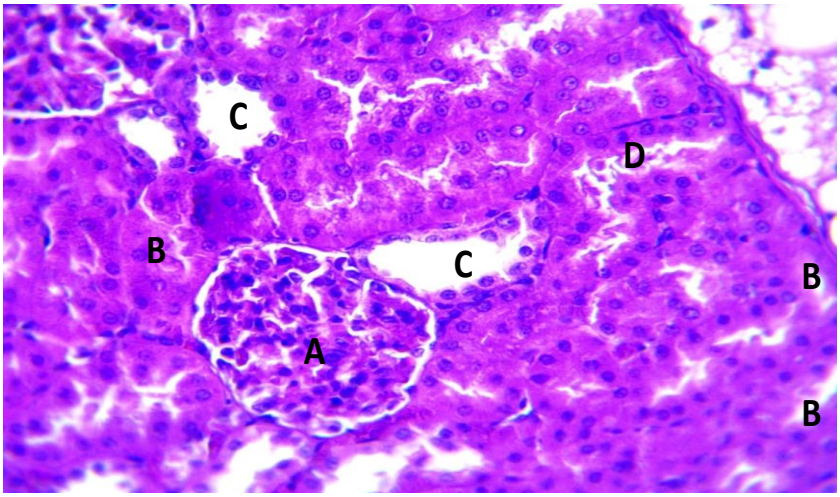

Figure 2. Standard group of males, renal cortex, normal shaped glomeruli (A), proximal (B), distal (C) convoluted tubules, normal shaped glomeruli, limited glomerular infiltrate (D) H2EX40.

We note from Figure (3) that the cortex of the kidney in female rats showed glomeruli with atrophy and shrinkage at the tip of Bowman's capsule with an increase in the expansion of the capsular space

and thickening of Bowman's capsule and the cavities of some tortuous tubules containing inflammatory fibrous infiltrate with the presence of epithelial cell debris in the cavity of other tubules, in addition to Loss of some epithelial cells lining the walls of some tubules.

In male rats, Figure (4), the cortex of the kidney showed atrophy and complete brittleness of some glomeruli, with an increase in the expansion of the capsular space around the glomeruli, in addition to the thickening of Bowman's capsule. An expansion was found in the lumen of some coiled tubules and the destruction of their epithelial cells, with the debris of epithelial cells in the lumen of other tubules. And the infiltration of large numbers of white blood cells around the glomeruli, coiled tubules, and some kidney arteries.

The results agreed with the findings of [17], as the rats were dosed orally with sodium nitrite at a concentration of 50 mg/kg of body weight for 19 days. The destruction of some urinary tubules and the epithelial cells lining the tubules, with the infiltration of some inflammatory cells.

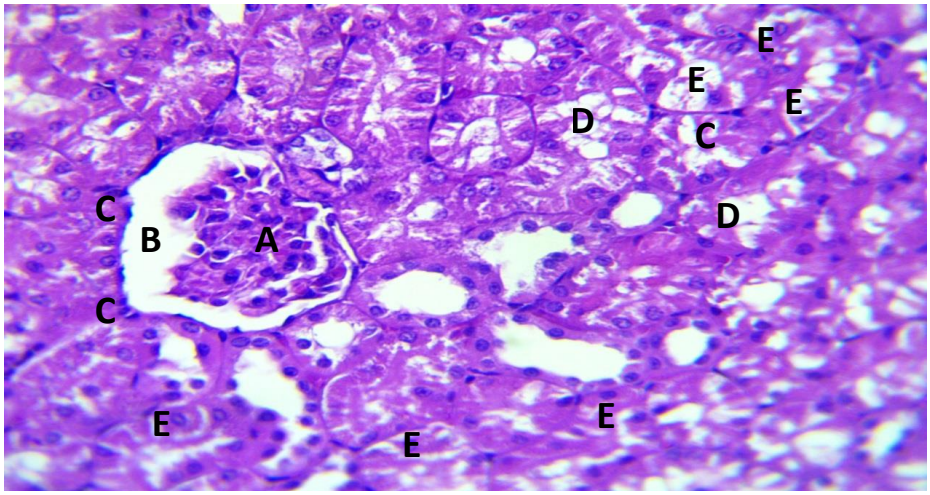

Figure 3. The group with renal failure without treatment for females (positive control) renal cortex, atrophy and shrinkage of glomerulus (A) capsular space expansion (B) thickening of Bowman's capsule (C) inflammatory fibrotic infiltrate (D) in the lumen of some tubules, cell debris in the lumen Other tubules (E) H2EX40.

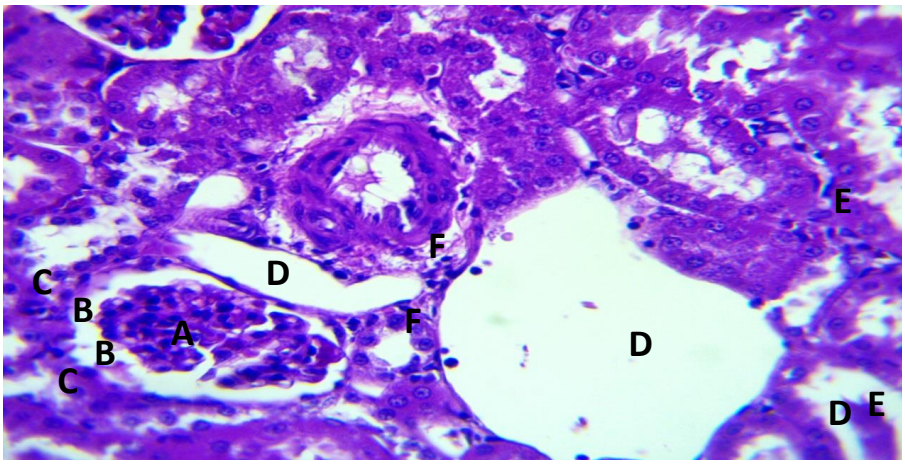

Figure 4. The group with renal failure without treatment for males (positive control) renal cortex, atrophy and complete lack of glomeruli (A) expansion of the capsular space (B) thickening of the wall of Bowman's capsule (C), expansion of the lumen of some tubules (D) debris of the wall of epithelial cells (E) In the lumen of the tubules, infiltration of white blood cells (F) around the arterioles of the kidney, glomerulus and tubules (H2EX40).

Figure (5) shows the cortex of the kidney of female rats, as it appeared normal, with the renal glomeruli having a standard structure and shape, in addition to the proximal and distal coiled tubules, with the presence of limited fibrous inflammatory oedema in a few of the lumen of the tubules.
In males, Figure (6) showed total embrittlement of the glomeruli of the kidney surrounded by the vast capsular space, the proliferation of some white blood cells on its surface, and the infiltration of inflammatory white blood cells as well, focally around the wall of Bowman's capsule, with an increase in the thickness of the colloidal bundles of the renal capsule, the tubules wrapped around the glomerulus in it. Degenerative epithelial cells within the lumen of the tubules with some proliferation of white blood cells among the outer capsular fibres.
The results agreed with a study [13], in which rats were orally dosed with a German extract of cress seeds at a concentration of 300 mg per kg of body weight before being induced with sodium nitrite at a concentration of 50 mg/kg of body weight for four weeks. The results showed a reduction in nephrotoxic effects. Harmful to nitrites, as the extract acts as a natural substance to mitigate changes in kidney function and oxidative damage caused by sodium nitrite in kidney tissues.

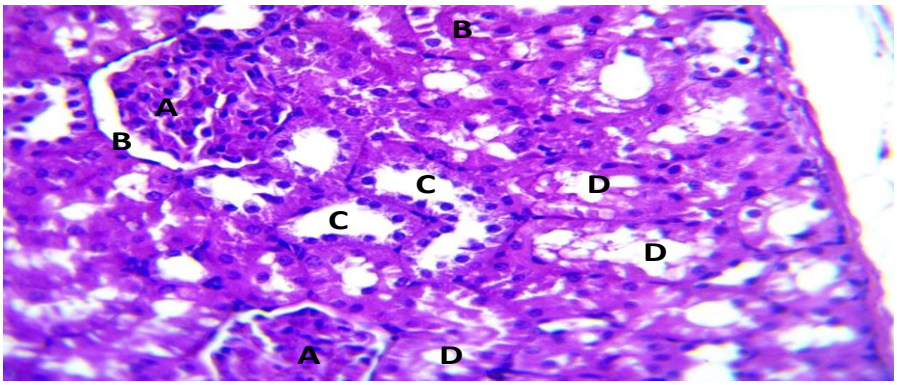

Figure 5. A group of female rats treated with water extract of cress seeds. The cortex of the kidney, glomeruli normal (A) proximal convoluted tubules (B) together and distal (C) a simple inflammatory fibrous infiltrate (D) in the lumen of some tubules (H2EX40).

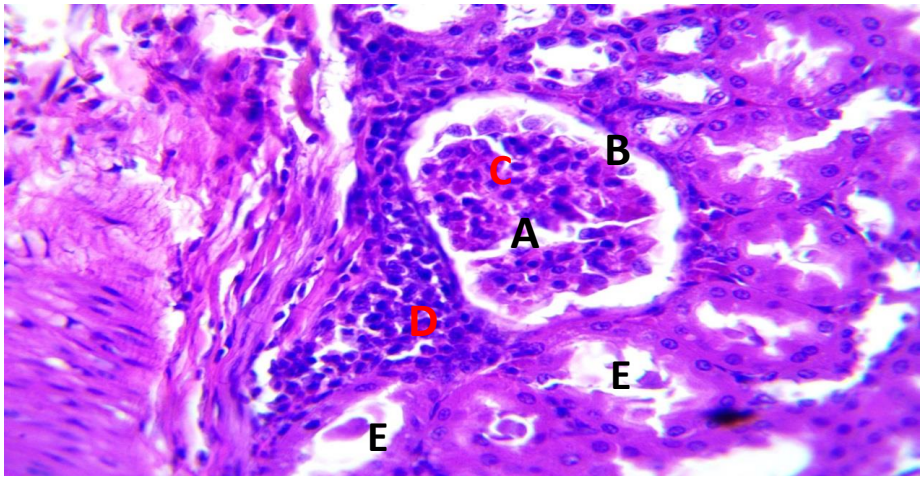

Figure 6. A group of male rats treated with water extract of cress seeds. The cortex of the kidney, total lobulation of the glomerulus (A) capsular dilatation (B) proliferation of white blood cells on the surface of the glomerulus (C) focal aggregation of white blood cells (D) around the glomerulus, dissected epithelial cells within the lumen of the tubules (E) (H2EX40).

Conclusions

The study concluded that oral administration of the aqueous extract of cress seeds to rats with renal failure led to an improvement in the levels of urea, creatinine, uric acid, and glomerular filtration of the rats' kidneys.

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Evaluation of the *Stagonosporopsis cucurbitacearum* Specialization Caused Gum Stem Blight Disease on Some Plants of the Cucurbitaceae

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Abstract. This study was carried out to show the specialization of some *S. cucurbitacearum* strains which causes gum stem blight disease, on four plant hsts of Cucurbitaceae family. The results of genetic analysis using nucleotide sequence analysis of the ITS regions of rDNA of S. *cucurbitacearum* isolates showed that the closest genetic distances were between S. *cucurbitacearum* strain Has.AA-16 (OP247683.1)and *S. cucurbitacearum* strain Has.AA-18 (OP247686.1), and both strains are closer to *S. cucurbitacearum* strain Has.AA-20 (OP247707.1) compared to the farthest genetic distance of 0.0202 for the isolate S. *cucurbitacearum* strain Has.AA-17 (OP247685.1). Isolates *S. cucurbitacearum* strain Has.AA-16 and *S. cucurbitacearum* strain Has.AA-20 showed a relative specialization in infecting melon, as it recorded the highest infection intensity of 88.32 and 88.03%, respectively, with the highest decrease in the vegetative indicators of melon plant, which included high Plant, vegetative and dry root weights, chlorophyll and leaf area, while the isolates *S. cucurbitacearum* strain Has.AA-17, *S. cucurbitacearum* strain Has.AA-18, and *S. cucurbitacearum* strain Has.AA-19 showed relative specialization in infecting cucumber, watermelon and squash plants, as it recorded the highest infection intensity of 87.9, 88.85, and 81.27%, respectively, with the highest decrease recorded in the vegetative indicators of these plants.

Keywords. Cucurbitaceae, gum stem blight disease, *Stagonosporopsis cucurbitacearum*, *Didymella bryoniae*, nucleotide sequence analysis, ITS.

1. Introduction

Stagonosporopsis cucurbitacearum (= *Didymella bryoniae*) is a fungal pathogen that affects plants, especially those in the cucurbit family such as cucumbers, melons, and squash. As it causes great losses if the conditions are appropriate, the disease also is known as black rot and its symptoms appear in all parts of the plant, the crown, stem, appendages, leaves, leaf petioles, flowers and fruits, and it infects the plant from the beginning of its life until the stage of production, which leads to wilting and death of the plant . Infected fruits develop sunken necrosis and rot, making them unfit for consumption [1,2]. The disease is spread through ascospores (sexual spores) in perithecia and conidia (asexual spores) in pycnidia and both of these spores are dispersed by rain/rain-splash and can live in the soil for several years. Control measures include crop rotation, use of disease-resistant varieties, and use of fungicides. Early detection and prompt action is crucial in preventing the spread of *Didymella*

bryoniae and reducing the damage it can cause to crop production[3]. The fungus *S. cucurbitacearum* has specialized in infecting plants in the cucurbit family and is a major disease threat to Cucurbitaceae crops worldwide [4]. Cucurbitaceae plants are particularly susceptible to *Didymella bryoniae* due to the to the possibility of penetration of this pathogen in the leaves and stems of these plants, and they provide a favourable environment for the growth and reproduction of the pathogen. In addition, the fungus can live in plant debris and soil for long periods of time, making it difficult to control, while other plant species may be susceptible to *Didymella bryoniae* infection, but Cucurbitaceae plants are the primary host and the one most affected by this pathogen [5]. Research confirmed that the gum blight disease caused by *D.bryoniae* (*syn.Stagonosporopsis spp*.) affects about 12 genera and 23 species of cucurbits and poses a threat to cucurbit production [3].

Symptoms of this disease appear from the beginning of the seedling season until it reaches the production stage. Symptoms initially include the crown area close to the soil surface, as transparent spots appear on it filled with water, to develop later and turn brown and then turn black. It spreads to the stems, crown and leaves, causing wilting of the leaves , with symptoms close to fusarium wilt disease [6]. Gum stem blight is a major disease of Cucurbitaceae plants caused by three distinct genotypes: *Stagonosporopsis* species, *S.cucurbitacearium* asexual (*Syn.D. bryoniae*), and *S.citrulli*, and *S. caricae*, Cucurbitaceae plants are the main host for species 1 and 2, while papaya is the main host for the latter species, *S.caricae* [7,8]. Many studies refer to another synonym, M. melonis (*Syn. D. bryoniae*), which infects cucumbers, melons, and watermelons [4, 9, 10]. Also in the Mediterranean regions of Asia, *S. cucurbitacearum* was diagnosed as the main pathogenic fungus of cucurbitaceae seedlings with gum stem blight, as it was shown to have the ability to reduce the quantity and quality of the cucurbitaceae plants yield [2, 11]. In another study, thirty-five isolates of *D. bryoniae* that cause symptoms of gummy stem blight on watermelon and squash varieties, in Florida and Georgia, were identified based on their phenotypic and molecular profile based on rDNA sequencing and RAPD which proved that all isolates were pathogenic, but there was genetic variation among the isolates through the different virulence of these isolates [12].

Gum stem blight spreads in several areas in Iraq, causing great losses in the cucurbitaceae plants. For the seriousness of this disease, the current study aimed to isolate and molecularly diagnose several strains of this fungus parasitizing on different cucurbitaceae plant to reveal the specialization of infection or not by these isolates.

2. Materials and Methods

2.1. Isolates of the Fungus (Didmella Bryonaie) S.cucurbitacearum

In this study, five isolates of the fungus that cause gum stem blight were used in some plants of the Cucurbitaceae family. The sources of these isolates were according to our previous study [13] as follows:

- *S.cucurbitacearum* strain Has.AA-16 (accession number OP247683.1) isolated from melon plants *Cucumis melo* from Samarra region.
- *S.cucurbitacearum* strain Has.AA-17 (accession number OP247685.1)from cucumber plants *Cucumis sativus* from Dujail region.
- *S.cucurbitacearum* strain Has.AA-18(accession number OP247686.1) from watermelon plants *Citrullus lanatus* in Al-Ishaqi region .
- *S.cucurbitacearum* strain Has.AA-19 (accession number OP247709.1)from Squash plants *Cucurbita pepo* from Tikrit region .
- *S.cucurbitacearum* strain Has.AA-20 (accession number OP247707.1) from melon plants *Cucumis melo* plants from the Al-Alam region.

2.2. Genetic Analysis

Mega 5.2 program was used to draw the Phylogenetic neighbor-joining tree based on the nucleotide sequences of the ITS region within the rRNA 5.8S gene.

2.3. Specialization Test of S.cucurbitacearum Isolates on some Plants of Cucurbitaceae Family

2.3.1. Preparing the Pathogenic Inoculum

The inoculum of *S. cucurbitacearum* isolates was prepared separately using the seeds of local millet *Panicum miliaceum* L. It was washed well and soaked for 6 hours, 100 g of millet seeds were put in 250 ml Cap. flasks, sterilized in autoclave at 121 C, 15 lb/in^2 for one hour, then left to cool, and each flask was inoculated with 5 fungal discs (diameter 5 mm) from the (age of 7 days), the flasks were incubated at 27 ± 2 ° C for 10 days, the flasks were shaken every 2 days to distribute the fungal inoculum [14].

2.3.2. Plant Cultivation and Inoculation with Pathogenic S.cucurbitacearum Strains

Five cucurbitaceae plants were cultivated on March 15, 2022 at the research station of the Plant Protection Department - College of Agriculture - University of Tikrit, with 50 plants for each type. The plants were inoculated with the pathogenic fungi at the age of three leaves per seedling by 5 g of the *S. cucurbitacearum* inoculum at a depth of 3 cm around the root of each seedling, then the plants were serviced by irrigation, weed hoeing and fertilization.

2.4. Studied Traits

2.4.1. Estimation of Plant Height and Root System Length

The length of the plant was measured from the soil surface to the apex of the plant using a tape measure, as well as measuring the length of the root system from the crown region to the last apex of the root [15].

2.4.2. Estimation of the Shoot and Dry Root Weight

After plants uprooting, were carefully washed with water in order to get rid of the suspended dust, and the vegetative system was separated from the root from the crown region, then the vegetative and root parts were dried in the electric oven at a 50 °C until weight stability, then weighed using a sensitive scale [15].

2.4.3. Determination of Chlorophyll Concentration

The total chlorophyll concentration was estimated by extracting chlorophyll in the leaves using acetone (80%), then reading the light absorption of the sample with a spectrophotometer at the wavelengths 663 and 645 nm, after which the total chlorophyll concentration was estimated [16].

2.4.4. Leaf Area

The leaf area was calculated using the Digimizer program, by taking the average of 10 fully developed leaves and calculating their area digitally to be multiplied by the total number of plant leaves to calculate the total leaf area.

2.4.5. The Infection Severity

The five-degree pathological index reported by [17] for gum stem blight was adopted.

- 0: no disease symptoms on both root and vegetative groups (plants are healthy)
- 1: yellowing of 1-25 leaves close to the crown area with light-colored spots
- 2: Yellowing of 26-50 leaves with brown spots and necrosis in the crown and stem.
- 3: Yellowing of 51-75 leaves with brown lesions on the leaves and stem and cracks on the stem.
- 4: Yellowing and death of 76-100 leaves with the spread of necrosis and cracks on the stem and the presence of brown or black gummy secretions.

The infection severity was estimated for all treatments according to the McKinney equation[18] , as follows:

$$\text{Infection severity} = \frac{\text{Sum(The number of infected plants at degree } 0 * 0 + \ldots\ldots + \text{the number of infected plants at degree } 6 * 6)}{\text{Total number of plants} * \text{highest score in the medical evidence}} \times 100$$

2.5. Statistical Analysis

The experiment was carried out using a completely randomized design, analysis of variance was performed using the program (SPSS), and treatment means were compared according to the Least Significant Deference (LSD) test, at a significant level of 0.05 [19].

3. Results

3.1. Genetic Analysis

Figure (1) showed the genetic tree of *S. cucurbitacearum* isolates. The figure showed that the closest genetic distances were between *S. cucurbitacearum* strain Has.AA-16 and *S. cucurbitacearum* strain Has.AA-18, both of which are closer to *S. cucurbitacearum* strain Has. AA-20 compared to the farthest genetic distance of 0.0202 for the *S. cucurbitacearum* strain Has.AA-17, and this refer to the isolates that infect watermelon and melon are more closely related to the isolates compared to the isolates that infect cucumber and squash.

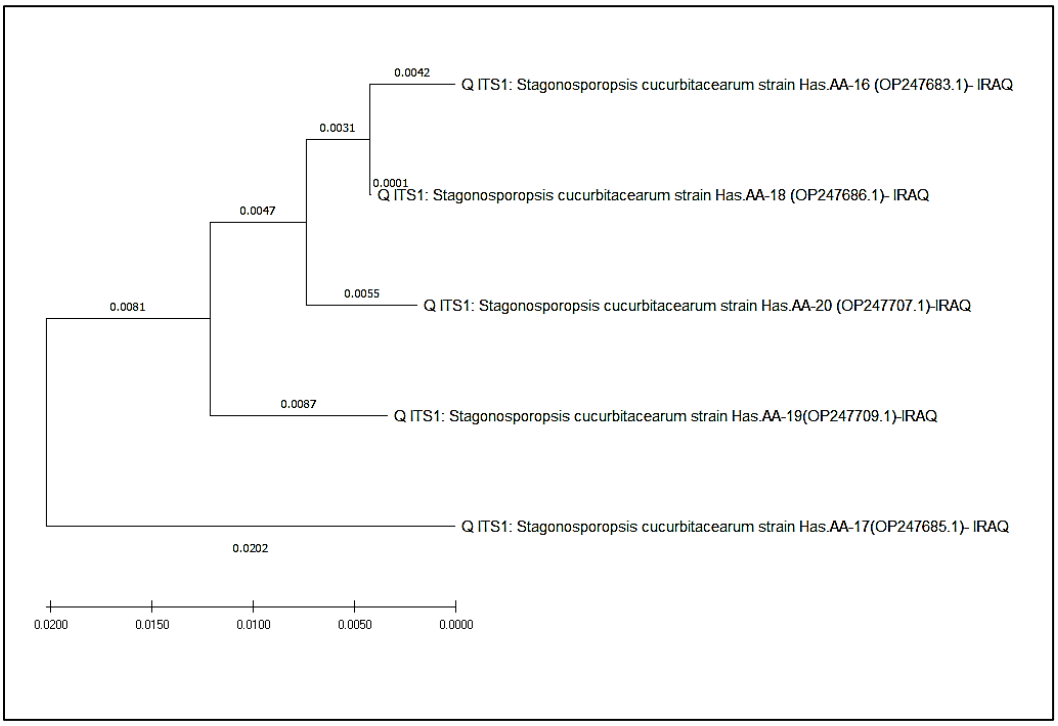

Figure 1. Phylogenetic neighbor-joining tree of the internal transcript spacer ITS of rDNA of five *S. cucurbitacearum* strains from various geographical region within Salah Al-din province , using Mega 5.2 software.

3.2. Effect of S.cucurbitacearum Isolates on Some Parameters of the Plant Vegetative Growth

3.2.1. Shoot System Height

Table (1) showed the effect of 5 isolates of *S. cucurbitacearum* on the shoot height of 4 plant species, the *S. cucurbitacearum* strain Has.AA-16 showed more effect on melon as it reached the lowest height of 61.54 cm, while *S. cucurbitacearum* strain Has.AA-17 showed the highest effect on cucumber plants, as the lowest plant height was 63.65 cm.

The strains Has.AA-18, Has.AA-19 and Has.AA-20 were recorded the highest effect on the lowest height of plants; watermelon, squash, and melon had the lowest height of the shoots, reached 61.11, 51.64, and 60.05 cm, respectively, compared to the healthy cucumber, squash, watermelon and melon plants, which reached 158.06, 147.28, 151.4, and 144.91 cm, respectively.

Table 1. Effect of *S. cucurbitacearum* strains on Shoot system height (cm) of four plant hosts.

***S. cucurbitacearum* strains**	**Host plant**				
	Cucumber	**Squash**	**Watermelon**	**Melon**	**Average of fungal strains**
S. cucurbitacearum strain Has.AA-16	133.65	82.05	78.33	61.54	88.89
S. cucurbitacearum strain Has.AA-17	63.65	65.17	111.06	128.39	92.07
S. cucurbitacearum strain Has.AA-18	124.72	94.11	61.11	79.12	89.77
S. cucurbitacearum strain Has.AA-19	139.68	51.64	101.21	83.10	93.91
S. cucurbitacearum strain Has.AA-20	139.04	81.11	77.37	60.05	89.39
Control (Healthy plants)	158.06	147.28	151.4	144.91	150.41
Average of Host plant	126.47	86.89	96.75	92.85	
$LSD_{0.05}$	Fungal strains=3.11 , Host plant= 5.43, Fungal strains× Host plant=7.06				

3.2.2. Root System Length

Table (2) showed the effect of *S. cucurbitacearum* isolates on the length of the root system of tested plants, where the *S. cucurbitacearum* strain Has.AA-16 showed more effect on melon, as the minimum length of the root was 7.61 cm., followed by the strains, Has.AA-17 and Has.AA-18, which showed the highest effect on cucumber and watermelon root length, as the lowest root length reached 8.66 and 5.45 cm, respectively. As for the strains Has.AA-19 and Has.AA-20, they recorded the highest effect on the lowest root length of squash and melon plants, with a minimum length of 7.34 and 7.8 cm, respectively, compared to the root length of healthy cucumber, squash watermelon and melon plants, which reached 57.33, 28.46, 27.78, and 25.51 cm, respectively.

Table 2. Effect of *S. cucurbitacearum* strains on Root system length (cm) of four plant hosts.

S. cucurbitacearum strains	**Host plant**				
	Cucumber	**Squash**	**Watermelon**	**Melon**	**Average of fungal strains**
S. cucurbitacearum strain Has.AA-16	22.03	11.77	7.97	7.61	12.35
S. cucurbitacearum strain Has.AA-17	8.66	24.95	23.83	21.06	19.63
S. cucurbitacearum strain Has.AA-18	22.72	11.12	5.45	9.08	12.09
S. cucurbitacearum strain Has.AA-19	19.04	7.34	15.82	13.22	13.86
S. cucurbitacearum strain Has.AA-20	21.51	12.43	10.39	7.8	13.033
Control (Healthy plants)	25.33	28.46	27.78	25.51	26.77
Average of Host plant	19.88	16.01	15.21	14.05	
$LSD_{0.05}$	Fungal strains=1.34 , Host plant=1.46 , Fungal strains× Host plant=2.33				

3.2.3. Dry Vegetative Weight

The results listed in Table (3) showed the effect of *S. cucurbitacearum* strains on the dry vegetative weight of cucumber, squash, watermelon and melon plants, the *S. cucurbitacearum* strain Has.AA-16 showed more effect on melon, as the lowest dry vegetative weight was 40.31. The strains Has.AA-17, Has.AA-18, Has.AA-19 and Has.AA-20 recorded the highest effect on cucumber, watermelon, squash and melon plants, as the lowest dry vegetative weights were 39.92, 41.06, 45.20 and 43.78 g, compared to 69.67, 93.88, 83.71 and 96.73 g in these healthy plants, respectively.

Table 3. Effect of *S. cucurbitacearum* strains on dry vegetative weight (g) of four plant hosts.

***S. cucurbitacearum* strains**	**Host plant**				
	Cucumber	**Squash**	**Watermelon**	**Melon**	**Average of fungal strains**
S. cucurbitacearum strain Has.AA-16	64.11	60.30	42.22	40.31	51.74
S. cucurbitacearum strain Has.AA-17	39.92	69.02	74.25	82.05	66.31
S. cucurbitacearum strain Has.AA-	65.87	55.86	41.06	47.73	52.63

S. cucurbitacearum strains	Host plant				
	Cucumber	**Squash**	**Watermelon**	**Melon**	**Average of fungal strains**
18					
S. cucurbitacearum strain Has.AA-19	62.78	45.20	58.66	51.90	54.64
S. cucurbitacearum strain Has.AA-20	66.43	62.00	44.68	43.78	54.22
Control (Healthy plants)	69.67	83.71	93.88	96.73	85.99
Average of Host plant	61.46	62.68	59.13	60.42	
LSD $_{0.05}$	Fungal strains=1.23 , Host plant=3.04 , Fungal strains× Host plant=5.10				

3.2.4. Dry Root Weight

Table (4) showed that the *S. cucurbitacearum* strain Has.AA-16 showed more effect on melon, as the lowest dry root weight was 26.66 g, while the strains Has.AA-17 and Has.AA-18 showed the highest effect on cucumber and watermelon plants, as the lowest dry root weight reached 20.84 and 25.47 g, respectively, on the other hand, the strains Has.AA-19 and Has.AA-20 had the highest effect on the lowest weight dry root of squash and melon with the lowest weights 23.33 and 27.04 g, respectively, compared to the dry root weights 48.82, 45.08, 65.87, 67.66 g of the healthy cucumber, squash, watermelon and melon plants, respectively.

Table 4. Effect of *S. cucurbitacearum* strains on dry root weight (g) of four plant hosts.

S. cucurbitacearum strains	Host plant				
	Cucumber	**Squash**	**Watermelon**	**Melon**	**Average of fungal strains**
S. cucurbitacearum strain Has.AA-16	40.54	37.75	32.82	26.66	34.44
S. cucurbitacearum strain Has.AA-17	20.84	38.78	51.16	61.53	43.08
S. cucurbitacearum strain Has.AA-18	39.68	36.46	25.47	32.17	33.45
S. cucurbitacearum strain Has.AA-19	44.04	23.33	42.09	27.84	34.33
S. cucurbitacearum strain Has.AA-20	41.56	35.72	31.70	27.04	34.01
Control (Healthy plants)	48.82	45.08	65.87	67.66	56.86
Average of Host plant	39.25	36.19	41.52	40.48	
LSD $_{0.05}$	Fungal strains= 1.61, Host plant= 3.41, Fungal strains× Host plant= 5.78				

3.3. Leaf Area

Table (5) showed the effect of 5 isolates of *S. cucurbitacearum* on the leaf area of the tested plant, the *S. cucurbitacearum* strain Has.AA-16 showed more effect on melon, as the leaf area reached 446.09 cm^2 / plant, while the strains; Has.AA-17, Has.AA-18, Has.AA-19 and Has.AA-20 had a higher effect on cucumber, watermelon, squash and melon plants, as the lowest leaf area was 761.43, 433.65 and 380.27, 420.47 cm^2/plant compared to the higher leaf area in the healthy cucumber, squash, watermelon and melon resulting in 3678.73, 3566.41, 3150.53, and 3472.33 cm^2 / plant, respectively.

Table 5. Effect of *S. cucurbitacearum* strains on the leaf area (cm^2/ plant) of four plant hosts.

S. cucurbitacearum strains	Host plant				
	Cucumber	**Squash**	**Watermelon**	**Melon**	**Average of fungal strains**
S. cucurbitacearum strain Has.AA-16	2866.87	2767.02	1843.65	446.09	1980.91
S. cucurbitacearum strain Has.AA-17	761.43	871.40	2028.26	2320.21	1495.33
S. cucurbitacearum strain Has.AA-18	2745.43	2573.50	433.65	1863.54	1904.03

S. cucurbitacearum strains	Host plant				
	Cucumber	**Squash**	**Watermelon**	**Melon**	**Average of fungal strains**
S. cucurbitacearum strain Has.AA-19	2888.66	380.27	2750.32	1933.46	1988.18
S. cucurbitacearum strain Has.AA-20	2882.07	2709.43	1826.28	420.47	1959.56
Control (Healthy plants)	3678.73	3566.41	3150.53	3472.33	3467
Average of Host plant	2637.2	2144.67	2005.45	1742.68	
$LSD_{0.05}$	Fungal strains= 37.74, Host plant=81.57 , Fungal strains× Host plant=95.29				

3.4. Chlorophyll Content

The results listed in Table (6) showed that the *S. cucurbitacearum* strain Has.AA-16 had a greater effect on melon, as the lowest concentration of chlorophyll was about 1.10 mg/g lea., followed by the strains Has.AA-17 and Has.AA-18, which showed the highest effect on cucumber and watermelon, as the lowest concentration of chlorophyll reached 1.05 and 1.14 mg/g leaf, respectively. The highest effect on the lowest concentration of chlorophyll in squash and melon plants were 0.89 and 0.83 mg/g leaf, when infected with strains Has.AA-19 and Has.AA-20 respectively. The higher chlorophyll concentrations were 3.66, 3.21, 3.81 and 3.16 (mg/g leaf) in the healthy cucumber, squash, watermelon and melon leaves, respectively.

Table 6. Effect of *S. cucurbitacearum* strains on the Chlorophyll content (mg/g leaf) of four plant hosts.

S. cucurbitacearum strains	Host plant				
	Cucumber	**Squash**	**Watermelon**	**Melon**	**Average of fungal strains**
S. cucurbitacearum strain Has.AA-16	1.74	1.55	1.46	1.10	1.46
S. cucurbitacearum strain Has.AA-17	1.05	2.33	2.38	2.54	2.08
S. cucurbitacearum strain Has.AA-18	1.74	1.46	1.14	1.31	1.41
S. cucurbitacearum strain Has.AA-19	1.93	0.89	1.67	1.43	1.48
S. cucurbitacearum strain Has.AA-20	1.87	1.67	1.44	0.83	1.45
Control (Healthy plants)	3.66	3.21	3.81	3.16	3.46
Average of Host plant	1.99	1.85	1.98	1.73	
$LSD_{0.05}$	Fungal strains=0.23 , Host plant= 0.31, Fungal strains× Host plant=0.43				

3.5. The Infection Severity

Table (7) showed the effect of *S. cucurbitacearum* strains on the infection severity of 4 plants related to the Cucurbitaceae family. *S. cucurbitacearum* strains; Has.AA-16 and Has.AA-20 were very virulent on the melon as the highest infection severity reached 88.32 and 88.03%, respectively, while the strains Has.AA-17, Has.AA-18 and Has.AA-19 were virulent on the cucumber, watermelon and squash as the highest infection severity reached 87.90, 88.85 and 81.27%, respectively.

Table 7. Effect of *S. cucurbitacearum* strains on the infection severity (%) of four plant hosts.

S. cucurbitacearum strains	Host plant				
	Cucumber	**Squash**	**Watermelon**	**Melon**	**Average of fungal strains**
S. cucurbitacearum strain Has.AA-16	61.08	68.66	83.89	88.32	75.49
S. cucurbitacearum strain Has.AA-17	87.90	73.76	53.22	47.57	65.61
S. cucurbitacearum strain Has.AA-	61.61	67.23	88.85	84.93	75.66

S. cucurbitacearum strains	Host plant				
	Cucumber	**Squash**	**Watermelon**	**Melon**	**Average of fungal strains**
18 *S. cucurbitacearum* strain Has.AA-19	70.11	81.27	73.66	79.12	76.04
S. cucurbitacearum strain Has.AA-20	63.33	71.05	73.87	88.03	74.07
Control (Healthy plants)	0	0	0	0	0
Average of Host plant	57.34	60.33	62.25	64.66	
$LSD_{0.05}$	Fungal strains=2.63 , Host plant= 2.21, Fungal strains× Host plant= 3.55				

4. Discussion

Through the results of this study, it was found that the isolates infecting watermelon and melon were more closely related to the isolates compared to the isolates infecting cucumber and squash, while *S. cucurbitacearum* strain Has.AA-17 was the farthest genetically from the rest of the tested *S. cucurbitacearum* strains according to the analysis of the genetic tree, although there is no absolute specialization of *S. cucurbitacearum* strains, but it has the highest effect in reducing vegetative growth parameters such as plant height, root length, dry weight of shoot and root systems, leaf area and chlorophyll cntent with high infection severity were recorded in melon plants by the *S. cucurbitacearum* strain Has.AA-16 and *S. cucurbitacearum* strain Has.AA-20.

It also recorded the lowest decrease in those vegetative parameters with the highest increase in the infection severity in cucumber, watemelon and squash when these plants were infected with *S. cucurbitacearum* strain Has.AA-17, *S. cucurbitacearum* strain Has.AA-18 and *S. cucurbitacearum* strain Has. AA-19. This indicates that there is a relative specialization of of the *S. cucurbitacearum* strains, and the evidence for this is that the primary isolation of these strains were from Cucurbitaceae family plants that showed the highest infection severity and the lowest vegetative growth parameters. The results of this study are consistent with respect to the variation in virulence of different strains of *S. cucurbitacearum*, which causes gum stem blight disease[11].

In the nucleotide sequence analysis of the ITS regions of rDNA, [20] clustered the isolates of the fungus *D. bryoniae*, which was later identified as *S. cucurbitacearum* into four groups according to their virulence to the plant host and according to the selected geographical regions in the United States of America from which they were isolated, as the analyzes placed the groups of this fungus RG-I and RG-11 in a common strain group close to RG-IV, with a higher genetic distance to these groups, with RG-III that placed separately as belonging to other fungi (Phoma spp.). Such a relative specialization of the same groups of fungi was also distinguished by the researcher [21], but using AFLP analysis. Babu [12] mentioned that these *D. bryoniae* strains can be distinguished through their virulence on the plant host, as well as molecular genetics analyzes such as Nucleotide sequence analysis of the ITS, RFLP analysis, and RAPD. Using ITS analysis, Babu [12] also showed the different American and Chinese strains were distinguished as being the most virulent on watermelon among the cucurbitaceae.

The relative specialization of *S. cucurbitacearum* strains, which is unique to this study, may be due to a genetic compatibility and the adaptation between the nutritional needs of the pathogen that it derives through parasitism and the components of the plant host tissues such as fibers, sugars, proteins, vitamins, etc., in addition to physiological and genetic factors. This is similar to the specialization of the phytopathogenic fungus *Fusarium oxysporum*, which causes vascular wilt diseases on its different hosts [22,23].

Conclusion

The present study showed The relative specialization of *S. cucurbitacearum* strains towards the various plants within the cucurbitaceae family. *S. cucurbitacearum* strains; Has.AA-16 and Has.AA-20 were very virulent on the melon, while the strains Has.AA-17, Has.AA-18 and Has.AA-19 were virulent on the cucumber, watermelon and squash. The low growth parameters of these plants and the high infection severity with these *S. cucurbitacearum* strains corresponded to a large extent with the analysis of the phylogenetic tree based on nucleotide sequence analysis of the ITS regions of rDNA.

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Evaluation of the Pleurotin Activity Extracted from the Oyster Mushroom *Pleurotus* spp. and its Compatibility with some Chemical Fungicides in Inhibiting the Growth of the Phytoathogenic Fungi *Rhizoctonia solani* and *Alternaria alternata*

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Abstract. This study was conducted in the College of Agriculture, University of Tikrit, aimed to evaluate the efficiency of the edible mushroom *Pleurotus* spp. in producing the antibiotic pleurotin. The study included an evaluation of the efficiency of five *Pleurotus* spp. and their ability to produce pleurotin. The results showed a variation in the effect of the culture medium type on the one hand, and the type of supplements materials on the other hand, on the concentration of pleurotin, as both the supplements; crushed sunflower seeds and wheat bran gave the highest concentration of pleurotin in all tested media compared to the control, the highest production of pleurotin was 17.71, 17.3, and 20.54 and 19.48 mg/L by *P. cornucopiae* cultured on wheat straw, corn cobs, a mixture of (wheat straw + rice husks) and a mixture of (barley straw + rice husks) supplemented with 5% sunflower seed powder, while the results showed that no pleurotin was recorded when supplemented with crushed soybeans for all tested media as a result of not growth of all *Pleurotus* spp. in present this supplement. Among 5 liquid media, *P. cornucopiae* recorded the highest production of pleurotin on Malt extract broth medium, reaching 40.02 mg/L, and it increased to 49.48 mg/L when this medium supplemented with 1% of crushed palm fibers. Pleurotin purified from oyster mushrooms in this study showed its compatibility with the fungicides Othilotop and Sabithane, as it achieved 100% inhibition of the pathogenic fungi, *Rhizoctonia solani* and *Alternaria alternata*, while it was less compatible with the Folio Gold, as the inhibition percentage of these fungi reached 80 and 60.3%, respectively. From these results, *P. cornucopiae* among five of *Pleurotus* spp. showed its superiority by producing the highest pleurotin concentration after fruiting in all tested media.

Keywords. Oyster mushroom, *Pleurotus* spp, Pleurotin, Fungicides, *Rhizoctonia solani*, *Alternaria alternate.*

1. Introduction

Oyster mushrooms, *Pleurotus* spp. were produced as an important source of food, including proteins, carbohydrates, minerals and vitamins, in addition to its valuable medical benefits [1] as it is characterized by containing effective compounds that contain antioxidants and antimicrobial properties and strengthens the immune system against carcinogens, tumors, heart diseases and

allergies [2,3] such as pleuran [5,4], anti-microbial and anti-cancer, pleurotin [6,7] , polysaccharides, proteins associated with complex sugars that have the ability to dissolve in water [8], in addition to the Mannan and β-glucans [9], antioxidants [10] and lavostatin and ergosterol that reduce cholesterol in human [11]. Pleurotin has the ability to produce antibacterial and antifungal agents with reduction of bacteria and fungi toxins therefore, these agents have been used to control fungal and bacterial infections [12]. Pleurotin, an antibiotic from naphthoquinone, one of the most important secondary metabolites extracted from some oyster mushrooms such as *Pleurotus griseus* as well as *Hohenbruehelia geogenius* and *Hohenbruhelia atrocaerulea*, which discovered for the first time by [13] as a substance produced from oyster mushrooms *Pleurotus griseus*, which is toxic to Gram-positive bacteria [13, 14], and it is an effective antibiotic against many types of bacteria and fungi. [15] indicated that pleurotin prevented the growth of dermatophyte fungi such as; *Trichophyton mentagrophytes* and *Candida albicans* in the laboratory, and against cancerous diseases as it inhibits the enzyme system responsible for cancerous tumors thioredoxin-thioreductase [16]. Some oyster mushrooms grown on different mediums lead to the production of different concentrations of pleurotin. [16] used several media, including Robbins medium, Potato dextrose broth (PDB), Malt medium extract, yeast extract, Sing medium, Yeast-malt broth, Soy glucose starch medium (SGSM) and Glucose sucrose fructose medium (GSF). Robbins' medium superior on the rest mediums in pleurotin production as it reached 440 mg/L followed by 340 m/L in PDB.

Rhizoctonia solani is an important and widespread soil fungus and one of the most important causes of seed rotting diseases and seedling death. It spreads all over the world and has a wide family range, which reaches more than 142 plant species belonging to 125 genera belonging to different plant families such as Solanaceae, Leguminosae, Asteraceae, Crassulaceae, and Brassicaceae families, as well as ornamental plants and trees [17], causing several diseases in different stages of plant growth, the most important of which are: seed rot diseases, seedling death, root and crown rot in tomatoes, wilting and black crust in potatoes, root and crown death, root and stem rot in bean, and death Stem of cloves and seedling drop disease on eggplant [18, 19]. *Alternaria alternate* belongs to a widespread opportunistic fungi and is a powerful foliar pathogen that damages host tissues by reducing the ability to photosynthesis, this fungus remains in stored products leading to latent infections that penetrate the tissues and remain dormant then infects the crops in suitable conditions [20]. *Alternaria* sp. has the ability to infect a wide range of plants and produce toxins, which are secondary metabolic that contribute to the invasion of plant tissue, however, severe infection leads to plant death and *Alternaria* spp. infected about 380 plant hosts and caused early blight disease, *Alternaria* spots which causing destructive spotting of the plant leaves, flowers and fruits [21]

For the importance of *Pleurotus* spp. and the lack of studies at the country level in the field of extracting antibiotics from it, such as pleurotin, and the difficulty of controlling the pathogenic fungus, *R.solani* because of its presence in the soil for a long time and formed of sclerotia that are characterized by their resistance to inappropriate conditions, in addition to reducing the use of chemical fungicides that are dangerous to humans and their environment, this study aimed to evaluation of the efficiency of five oyster mushroom *Pleurotus* spp. in the production of the antibiotic Pleurotin on different media and test its activity in the inhibition of the phytopathogenic fungi *Rhizoctonia solani* and *Alternaria alternate* in vitro with study of its compatibility with some chemical fungicides.

2. Materials and Methods

The experiments were carried out in the laboratories and the pioneer mushroom production farm in the College of Agriculture - Tikrit University, in the production cycle for the period from 1/18/2022_5/20/2022.

2.1. Oyster Mushroom Pleurotus spp.

Five of oyster mushrooms were used in the present study, including *Pleurotus ostreatus* strain Has.AA-10, *P. floridanus* strain Has.AA-12, *P. cornucopiae* strain Has.AA-13, *P. cystidiosus* strain Has.AA-11, and *P. eryngii* var. *ferulae* strain Has.AA-14 isolated from the Iraqi environment which diagnosed phenotypically and molecularly and registered in the global gene bank in NCBI with

accession numbers ON834664.1, ON834665.1, ON834668.1, ON834666.1 and ON834667.1, respectively [22].

2.2. Isolation and Identification of the Pathogenic Fungi from Infected Bean (Vicia faba)

2.2.1. Isolation of Rhizoctonia Solani from Roots

The pathogenic fungi were isolated from broad bean plants that showed symptoms of root and stem base rot disease. The stem area close to the soil surface and the roots were separated from the rest of the plant parts at a height of 5 cm above the crown area. Roots were washed in running water for 30 minutes, cut into small pieces 0.5-1 cm long and sterilized with sodium hypochlorite (NaOCL) at a concentration of 1% for 3 minutes, then washed with sterile water three times and dried on filter paper Whatman-N0.4, 5 pieces were transferred to sterile Petri dishes with a diameter of 9 cm containing 15-20 ml of PDA culture medium. 5 dishes were made for each sample then the dishes were incubated at 25 ± 2 °C for 3-5 days [10]. The fungus was diagnosed phenotypically based on the characteristics of the fungal colony, such as the shape, color, the nature of its mycelium, and the structures like sclerotia using the taxonomic key [23]. Then the fungus identified on the species level by nucleotide sequence analysis of the ITS regions of rDNA.

2.2.2. Isolation of Alternaria Alternata from Infected Bean Leaves

Samples of broad bean leaves showed symptoms of Alternarial spot, represented by brown spots surrounded by a yellow halo. The leaves were washed with running water to remove dust, then left for a short period to dry. These parts were cut into small pieces of 0.5 - 1 cm long and sterilized with sodium hypochlorite solution, washed, transferred to PDA medium and incubated as in *Rhizoctonia solani*, then the fungus identified on the species level by nucleotide sequence analysis of the ITS regions of rDNA.

2.3. Molecular Identification

The pathogenic fungi *R. solani* and *A. alternata* were diagnosed to the species level according to the molecular method based on the analysis of nucleotide sequences of the 5.8 S rRNA gene. All steps including Genomic DNA Isolation, Polymerase chain reaction (PCR) using primer pair, Forward 5′-TCCGTAGGTGAACCTGCGG -3′ Reverse 5′ TCCTCCGCTTATTGATATGC-3 , Agarose gel electrophoresis for both genomic DNA and PCR products and Nucleotide sequencing analysis were carried out according previuos study [24].

2.4. Cultural Medium for Isolation of the Pathogenic Fungi

2.4.1. Potato Dextrose Agar (PDA)

The medium was prepared by dissolving 39 g of PDA powder (HiMedia- India) in 1000 ml of distilled water the medium was distributed in four 500 ml flasks, about 250 ml for each flask, then its nozzles were closed with tightly closed cotton plugs, and sterilized using by autoclave at 121C with pressure of 15 pounds/in^2. After that, cooled to 40 °C, then Chloramphinicol was added at a rate of 250 mg / L, then the medium was poured into Petri dishes and left to solidify.

2.4.2. Effect of Nutritional Media on the Production of Pleurotin

Six liquid media (HiMedia- India) were tested for the production of Pleurotin from Pleurotus spp.

2.4.3. Potato Dextrose Broth (PDB)

This medium was prepared following the company's recommendations, by dissolving 24 g of the medium into 1000 ml of distilled water, the medium was distributed in flasks (capacity of 500 ml) at the rate of 150 ml for each flask, closed using cotton plugs, then autoclaved at 121°C with a pressure of 1.5 kg / cm^2 for 15 minutes

2.4.4. Malt Extract Broth (MEB)

This medium was prepared by dissolving 15 g of the culture medium in 1000 ml of distilled water, distributed in flasks, sterilized as in PDB medium.

2.4.5. Corn Meal Broth (CNB)
This medium was prepared according to the manufacturer , by dissolving 50 g of corn powder in 1000 ml of distilled water, distributed in flasks, sterilized as in PDB medium.

2.4.6. Sabouraud Dextrose Broth (SDB)
This medium was prepared by dissolving 15 g of the culture medium in 1000 ml of distilled water, distributed in flasks, sterilized as in PDB medium.

2.4.7. Malt Yeast Extract Broth(MYEB)
MYEB was prepared by dissolving 16 g of the culture medium in 1000 ml of distilled water, distributed in flasks, sterilized as in PDB medium.

2.5. Preparation of the Pleurotus spp. Spawn
The oyster mushroom spawn was prepared according to the standard method reported by[25].

2.5.1. Cultivation of the Pleurotus spp.
The culture media including wheat straw, corn cobs, a mixture of (wheat straw + rice husks 1:1, w:w) and a mixture of (barley straw + rice husks 1:1,w:w), were used for production of *Pleurotus spp*. fruit bodies. These media were soaked in tap water overnight , then the excess water was removed and pasteurized in the pasteurization chmber at 80 °C for 60 minutes. After cooling, the media were inoculated with 3% of mushroom spawn (on the basis of the culture medium dry weight). The inoculated media were filled into nylon bags (18 × 28 cm) and incubated at 22 °C. For fruiting, the humidity in the incubation room was raised to 90-80% by spryer machin with continuous ventilation, the temperature was maintained at 16-18^{o}C [26].

2.6. Supplementation
The tested media were supplemented with organic supplements included, wheat bran, crushed sunflower seeds and crushed soybeans, which were mixed with 5% of the media. Pasteurization, spawning and incubation were done according to the previous method.
Pleurotin extraction from culture media after fruiting
Pleurotin was extracted from culture media after harvesting the fruit bodies of oyster mushrooms according to the standard method mentioned [23].

2.6.1. Pleurotin Production by Pleurotus spp. Growing on Liquid Media
The liquid media PDB, MEB, CNB, SDB and MYEB were inoculated with five oyster mushrooms *Pleurotus* spp. with transfer a disc (0.5 cm) from each fungus cultures grown on PDA (age of 7 days). three replications were used for each mushroom and incubated at 27 °C for 20 days, after completion of its growth in the liquid media. The cultures were filtered using Whatman No. 1 filter paper then sterilized with filtration using 0.4 microns Millipore filter [14].

2.6.2. Effect of Supplementation with some Plant Wastes on the Production of Pleurotin in the Liquid Media
According to the preliminary results that showed the medium MEB as the best medium that gave the highest production of pleurotin, this experiment was achieved by supplementation this medium with 11 of plant wastes powders (apple, Populus, berries, Olives, Apricots, Sider (Rhamnus) , Palm racemes, Palm fronds, Palm fiber, Eucalyptus and Albizia) these wastes were crushed in mortar until it disintegrated, then it was ground with electrical mill. MEB medium was separately supplemented with 1% of each plant powder, then sterilized with an autoclave at of 121 ^{o}C ,a pressure of 1.5 kg / cm for 30 minutes. After cooling, inoculated with *Pleurotus cornucopiae* , incubated and pleurotin extraction according to the previous method.

2.7. Effect of some Chemical Fungicides on the Growth of Pathogenic Fungi

This experiment was carried out to test the effect of the fungicides listed in Table (1) in addition to pure pleurotin on the inhibition of *A. alternata* and *R.solani*. These fungicides were prepared according to the concentration of each fungicide (Table 1) in the PDA medium, after being sterilized by autoclave and cooling to 40°C, chloramphinicol was added at a rate of 250 mg/L, then the fungicides were also added separately, the medium was poured into Petri dishes and left to solidify. The centre of each plate was inoculated with a piece of 0.5 cm in diameter of the pathogenic fungi colony. All plates were incubated at 25°C, after completion of fungal growth in the control treatment, the growth rate of the fungi grown in present of fungicides were calculated by taking the average of two perpendicular diameters. the percentage of growth inhibition was calculated [27].

Inhibition rate =The growth diameter rate in the comparison - the rate of the growth diameter in the treatment / the rate of the growth diameter in the treatment × 100

2.8. Compatibility of Chemical Fungicides with Pleurotin on the Growth of Pathogenic Fungi

The fungicides with the highest effect on the growth of pathogenic fungi were selected in a ratio of 1:1 with pleurotin to show the compatibility of half concentration of pleurotin with these fungicides. Fungicides were mixed with Pleurotin and mixed with the PDA medium. The dishes were inoculated, incubated with pathogenic fungi, as well as the rate of fungal growth inhibition was calculated according to the previous method.

Table 1. Pleurotin and fungicides, their active ingredient, concentrations and producing companies.

Fungicides	Active ingredient	Concentration (%)	Origin
Othilotop	Difenoconazole + Azoxystrobin	0.1	Probelta - Spain
Previcur Energy	Fosetyl-aluminium	0.3	Bayer-Germany
Swift	Carbendazim 50%	0.05	Agricim - Australia
Tachegarin	Hymexazol 40%	0.1	Nippon-soda Japan
Sabithane	Dinocap Myclobutanil	0.1	Dow Agrosciences-England
Folio Gold	Mefenoxam 3.75% + Chlorothalonil 50%	0.25	Syngenta - Switzerland
Topsin Wp	Thiophanate methyl 70%	0.1	Nippon-soda Japan
Switch	Cyprodinil 37.5% Fludioxonil25%	0.05	Syngenta - Switzerland
Mizeb Wp	Mancozeb 80%	0.1	European Union
Talman-compy	Metalaxyl Mancozeb	0.25	Spain
Pleurotin	Naphthoquinone	0.2	Purified in this study

2.9. Statistical Analysis

The experiments of this study were carried out in a completely randomized design (CRD). An analysis of variance was carried out according to the SPSS program, and the averages were compared according to the Least Significant Deference (L.S.D) at the ≤ 0.05 [28].

3. Results

3.1. Effect of Wheat Straw Medium and some Supplements on Pleurotin Production After Fruiting

The results listed in Table (2) showed the effect of wheat straw medium and some nutritional supplements on the concentration of pleurotin produced from oyster mushrooms *Pleurotus* spp. after fruiting. The presence of the sunflower seeds and wheat bran recorded the highest concentration of pleurotin compared with control in all tested media. The results of the table 2 showed that no pleurotin was recorded in the medium of wheat straw with soybean meal due to the lack of growth of all oyster

mushrooms. The results recorded the highest concentration of pleurotin in the fungus *P. cornucopiae*, which reached 17.71 mg/l grown in wheat straw medium supported with crushed sunflower seeds, followed by fungus *P. cystidiosus*, as the pleurotin concentration reached 15.83 mg/l in the same medium compared to the lowest concentration was 0.88 mg/L for *Pleurotus eryngii* var. *ferulae*

Table 2. Effect of wheat straw medium and some supplements on pleurotin production after fruiting.

Treatments	*Pleurotus* spp.					Average of treatments
	P. ostreatus	***P. floridanus***	***P. cornucopiae***	***P. cystidiosus***	***P. eryngii* var. *ferulae***	
Ws	8.57	10.06	12.79	11.11	0.88	8.68
Ws+ Sf	11.22	14.43	17.71	15.83	2.27	12.29
Ws+ Wb	10.19	13.37	15.38	14.57	2.06	11.11
Ws+ Sb	-	-	-	-	-	
Average of *Pleurotus* spp.	9.99	12.62	15.29	13.84	1.75	
$LSD_{0.05}$	Treatments=2.02 , *Pleurotus* spp.=1.05 , Treatments× *Pleurotus* spp.=2.46					

Ws = wheat straw, Sf = sunflower seed meal, Wb = wheat bran, Sb = soybean meal. Pleurutin was extracted from 10 g solid medium in 100 mL of ethyl acetate.

3.2. Effect of Corn Cobs Medium with some Supplementation on Pleurotin After Fruiting

It is noted from Table (3) the effect of the corn cobs with some nutritional supplements on the concentration of pleurotin produced from the oyster mushroom *Pleurotus* spp. after fruiting. Both supplements, crushed sunflower seeds and wheat bran, led to the highest concentration of pleurotin , The results showed that the highest concentration of pleurotin was recorded at 17.3 mg / L in the corn cobs cultivated with *P. cornucopiae* and supplemented with crushed sunflower seeds, followed by 15.42 mg / L for the fungus *P. cystidiosus* in the same medium, while the lowest concentration of pleurotin was 0.88 mg/L in *Pleurotus eryngii* var. *ferulae* grown in the corn cobs only.

Table 3. Effect of corn cobs medium with some supplementation on pleurotin after fruiting.

Treatments	*Pleurotus* spp.					Average of treatments
	P. ostreatus	***P. floridanus***	***P. cornucopiae***	***P. cystidiosus***	***P. eryngii* var. *ferulae***	
Cc	8.57	10.06	12.79	11.11	0.88	8.68
Cc+ Sf	10.81	14.02	17.3	15.42	1.86	11.88
Cc+ Wb	9.58	12.76	14.77	13.96	1.45	10.50
Cc+ Sb	-	-	-	-	-	
Average of *Pleurotus* spp.	9.65	12.28	14.95	13.50	1.40	
$LSD_{0.05}$	Treatments= 1.12, *Pleurotus* spp.= 1.03, Treatments× *Pleurotus* spp.=1.76					

Cc = Corn cobs, Sf = sunflower seed meal, Wb = wheat bran, Sb = soybean meal. pleurotin was extracted from 10 g solid medium in 100 mL of ethyl acetate.

3.3. Effect of Medium (Wheat Straw + Rice Husks) Medium and some Supplements on the Concentration of Pleurotin After Fruiting

Table (4) showed the effect of a mixture of (wheat straw + rice husks) and some nutritional supplements on the concentration of pleurotin produced from *Pleurotus* spp. mushrooms after fruiting. The results showed that the highest concentration of pleurotin reached 20.54 mg / L in the treatment of *P. cornucopiae* growing in the medium of (wheat straw + rice husks) supplemented with crushed sunflower seeds, followed by 18.66 mg / L for the fungus *P. cystidiosus* in the same medium, while the lowest concentration of pleurotin was 2.14 mg/L in *Pleurotus eryngii* var. *ferulae* grown in the unsupplemented (wheat straw + rice husks) medium.

Table 4. Effect of medium (wheat straw + rice husks) medium and some supplements on the concentration of pleurotin after fruiting.

Treatments	*Pleurotus* spp.					Average of treatments
	P. ostreatus	***P. floridanus***	***P. cornucopiae***	***P. cystidiosus***	***P. eryngii* var. *ferulae***	
Ws/Rh	9.83	11.32	14.05	12.37	2.14	9.94
Ws/Rh+ Sf	14.05	17.26	20.54	18.66	5.1	15.12
Ws/Rh+ Wb	12.13	15.31	17.32	16.51	4.0	13.05
Ws/Rh+ Sb	-	-	-	-	-	
Average of *Pleurotus* spp.	12.00	14.63	17.30	15.85	3.75	
$LSD_{0.05}$	Treatments=1.66 , *Pleurotus* spp.=2.07 , Treatments× *Pleurotus* spp.=3.04					

Ws/Rh ; wheat straw + rice husks.

3.4. Effect of Medium (Barley Straw + Rice Husks) Medium and some Supplements on the Concentration of Pleurotin After Fruiting

The results in table 5 showed that the highest concentration of pleurotin was recorded in the treatment of *P. cornucopiae* grown in the medium of (barley straw + rice husks) supplemented with crushed sunflower seeds, as it reached 19.48 mg / L, followed by 17.6 mg / L for *P. cystidiosus* in the same medium. compared to *Pleurotus eryngii* var. *ferulae*, which gave the lowest concentration of pleurotin amounted to 1.59 mg / L in the unsupplemented (barley straw + rice husks) medium .

Table 5. Effect of medium (barley straw + rice husks) medium and some supplements on the concentration of pleurotin after fruiting.

Treatments	*Pleurotus* spp.					Average of treatments
	P. ostreatus	***P. floridanus***	***P. cornucopiae***	***P. cystidiosus***	***P. eryngii* var. *ferulae***	
Bs/Rh	9.28	10.77	13.5	11.82	1.59	9.39
Bs/Rh + Sf	12.99	16.2	19.48	17.6	4.04	14.06
Bs/Rh + Wb	11.25	14.43	16.44	15.63	3.12	12.17
Bs/Rh + Sb	-	-	-	-	-	
Average of *Pleurotus* spp.	11.17	13.8	16.47	15.02	2.92	
$LSD_{0.05}$	Treatments=1.83 , *Pleurotus* spp.=1.22 , Treatments× *Pleurotus* spp.=2.22					

Bs/Rh; barley straw + rice husks

3.5. Effect of some Liquid Media on the Concentration of Pleurotin Produced from Oyster Mushroom Pleurotus spp.

The results listed in Table (6) showed that the highest concentration of pleurotin was recorded in the treatment of the fungus *P. cornucopiae* growing in MEB medium, reaching 40.02 mg / L, followed by 39.37 mg / L for the same fungus growing in PDB medium compared to *P. eryngii* var. *ferulae* which gave the lowest pleurotin concentration was 11.09 mg/L in SDB medium.

Table 6. Effect of some liquid media on the concentration of pleurotin produced from oyster mushroom *Pleurotus* spp.

Liquid media	*Pleurotus* spp.					Average of treatments
	P. ostreatus	***P. floridanus***	***P. cornucopiae***	***P. cystidiosus***	***P. eryngii* var. *ferulae***	
CMB	15.52	21.73	30.90	22.23	12.52	20.58
PDB	22.06	27.54	39.37	35.91	13.63	27.70
SDB	16.46	20.57	27.79	17.61	11.09	18.70
MEB	21.33	32.52	40.02	36.42	12.70	28.60
YEB	22.88	26.93	38.44	29.92	17.97	27.23
Average of *Pleurotus* spp.	19.65	25.86	35.30	28.42	13.58	
LSD $_{0.05}$	Media = 1.66, *Pleurotus* spp.=3.81 , Media × *Pleurotus* spp.= 4.79					

3.6. Effect of Malt Extract Medium Supplemented with some Types of Plant Wastes Powders on the Concentration of Pleurotin Produced by the Fungus Pleurotus Cornucopiae

Figure (1) showed the effect of malt extract medium supplemented with some types of plant wastes powders on the concentration of pleurotin produced from the fungus *P. cornucopiae.* The highest concentration of pleurotin was recorded in the fungus *P. cornucopiae* treatment, as it reached 49.48 mg/L in the palm fronds powder treatment. It was followed by 44.27 mg / L in the treatment of Sidr powder, then 30.25 mg / L in the treatment of apricot powder, compared to 8.08 mg / L in the treatment of eucalyptus powder for the same fungus.

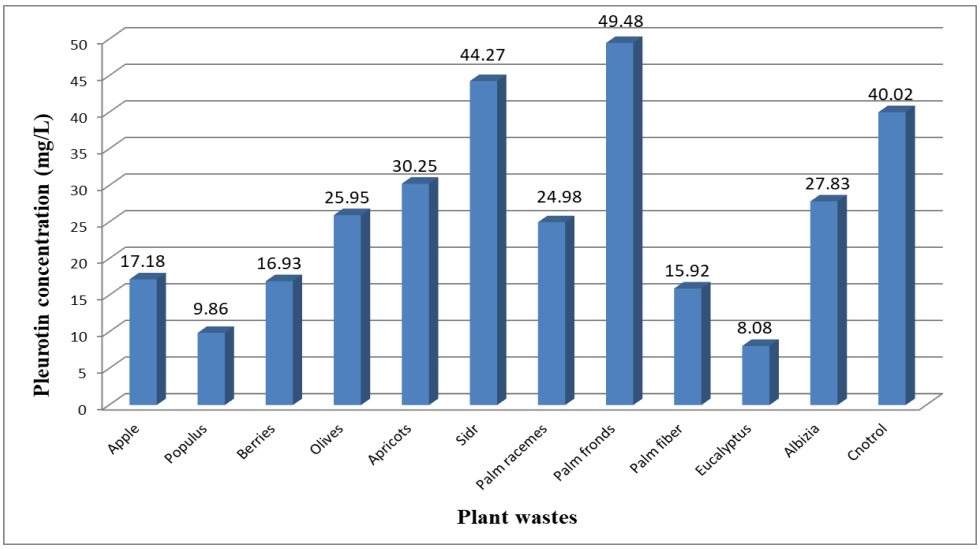

Figure 1. Effect of malt extract medium supplemented with some types of vegetable powders on the concentration of pleurotin produced from *Pleurotus cornucopiae* (LSD 0.05; 3.06).

3.7. Molecular Identification of the Pathogenic Fungi

Fungal isolates were identified at the species level using the nucleotide sequence analysis method. Figure (A2) showed the bands resulting from the electrophoresis of genomic DNA, while Figure (2B) showed the electrophoresis of the PCR product using a universal primer to amplify the 5.8S rRNA gene. The results of electrophoresis of the PCR product showed that the bands are 550 base pairs in size. Table showed the molecular diagnosis of these isolates to the species level, as the results indicated the *Alternaria alternata* strain Has.AA-9 matched 99.06% with *Alternaria alternata isolate ScA076* (from China) while *Rhizoctonia solani* strain Has.AA-15 matched 98.81% with *Rhizoctonia*

solani isolate BKJT9-1(from China). The isolated pathogenic fungi; Alternaria alternata strain Has.AA-9 and Rhizoctonia solani strain Has.AA-15 were registered in the Global Gene Bank at the NCBI site with the accession numbers ON834662.1 and ON921090.1, respectively.

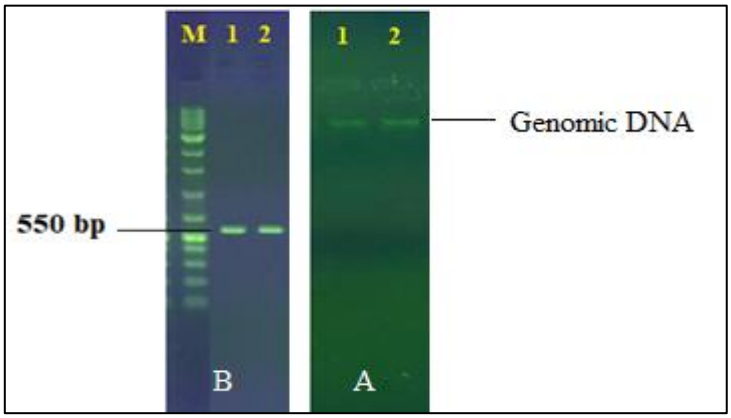

Figure 2. Gel electrophoresis of genomic DNA extraction from fungi, 1% agarose gel at 5 vol /cm for 1hour (A) , PCR product, 1.5% agarose gel at 5 vol /cm for 1hour (B).

Table 7. Molecular diagnosis of fungal isolates based on the percentage matching of the 5.8S rRNA gene sequences with fungal strains in the World Genebank.

The most compatible fungal species	Accession number	Country	Similarity (%)	Fungal strains recorded in the World Genetic Bank	Accession number of fungi registered in this study
Alternaria alternata isolate ScA076	ON796496.1	China	99.06	*Alternaria alternata* strain Has.AA-9	ON834662.1
Rhizoctonia solani isolate BKJT9-1	MN961664.1	China	98.81	*Rhizoctonia solani* strain Has.AA-15	ON921090.1

3.8. Effect of some Chemical Fungicides on the Growth of Pathogenic Fungi

Figure (3) showed the effect of some fungicides and pleurotin in inhibiting pathogenic fungi, *R. solani* and *A. alternata*, as the fungicides (Othilotop, Swift, Saithane, Folio Gold and Swich) recorded the highest inhibition rate for *R. solani*, which reached to 100%, respectively, while Tachegarin gave the lowest inhibition rate 69.6% to *R. solani*, , followed by Previcur Energy, Mizeb, Topsin and Talman-Compy, which reached to 83.6, 92, 91.5, 90.8%, respectively, compared to 92.7% for the treatment of pleurutin only. As for the pathogenic fungus *A. alternata*, Switch gave the highest inhibition rate of 100%, followed by Othilotop with a inhibition rate of 92.2%, while Topsin recorded the lowest inhibition rate for the pathogenic fungus *A. alternata*, which amounted to 20.6%, followed by Tachegarin with an inhibition rate 40.8% compared to 86.3% for the treatment of pleurotin only.

3.9. Compatibility of Chemical Fungicides with Pleurotin on the Growth of Pathogenic Fungi

Figure (3) showed the compatibility of pleurotin with the most efficient fungicides in inhibiting the pathogenic fungi, *R. solani* and *A. alternata*. The highest inhibition rates for both fungi were 100% in the treatments of (Othilotop+Pleurotin), (Sabithane+Pleurotin) and (Switht+Pleurotin) while the mixture of (Folio Gold+Pleurotin) gave the lowest inhibition rates for *R. solani* and *A. alternata* reached 80 and 60.3%, respectively.

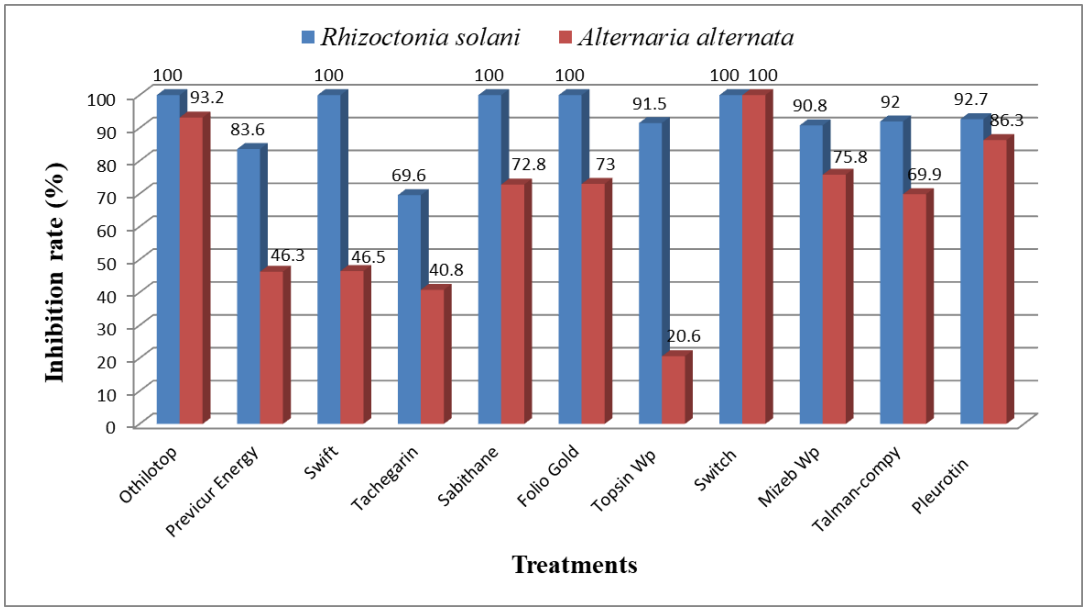

Figure 3. The effect of some pesticides and pleurotin in inhibiting the two pathogenic fungi, *R. solani* and *A. alternata* (LSD = 3.57).

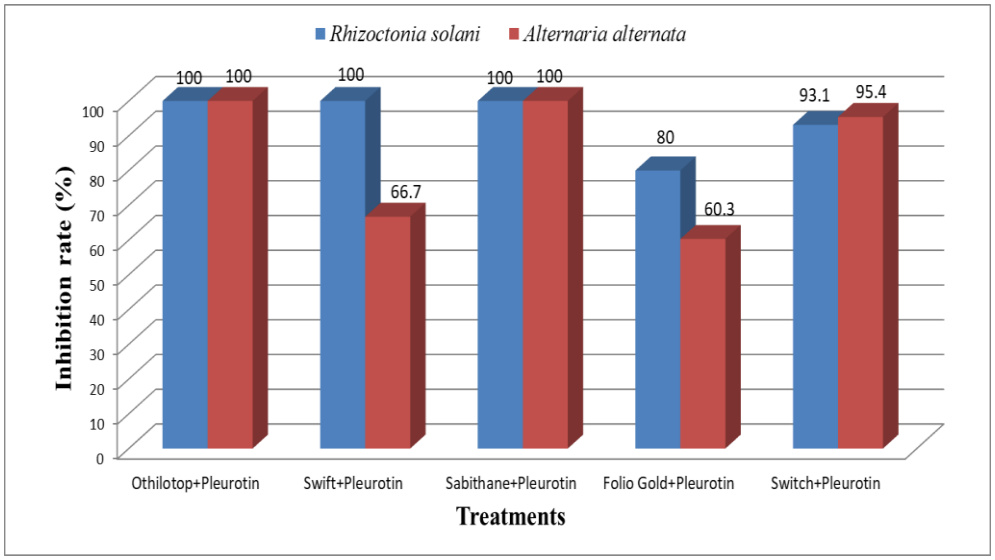

Figure 4. The effect of the compatibility of pleurotin with the most efficient fungicides in inhibiting the pathogenic fungi, *R. solani* and *A. alternata* (LSD = 3.11).

4. Discussion

The results of the effect of the culture media used in the production of pleurotin showed a variance in the effect of the type of culture medium on the concentration of pleurotin. This may be due to the fact that the components of the culture medium play an important role in the growth of fungus and the production of pleurotin, in addition, the difference in the chemical components of the solid medium such as cellulose, hemicellulose, lignin, proteins, lipids, minerals, etc., according to the nutritional requirements of the fungi, this reflected in all the bioactivities of the fungus. The results showed that the highest concentration of pleurotin was recorded in both crushed sunflower seeds and wheat bran in all the tested media compared to the unsupplemented media, while the results showed that no concentration of pleurotin was recorded in the crushed soybeans for all media as a result of the lack of growth of all oyster mushrooms *Pleurotus* spp., the reasons may be due the crushed soybean seeds were unsuitable medium for the growth of fungi due to its high nitrogen content, which is difficult for utilization by these mushrooms. The difference in the media and the different supplements lead to a

difference in the biological activity and metabolism of oyster mushrooms, then reflected in the production of metabolic compounds, including pleurotin. Our results agree with the study of [16], which indicated that the type of medium determines the production of pyloriutin by studying several media, including Robbins medium, (Potato dextrose broth (PDB), Malt extract medium, yeast extract, Sing medium, Yeast-malt broth, (Soy glucose starch medium (SGSM), Glucose sucrose fructose medium (GSF) among them, Robbins media outperformed the production of pleurotin over the rest of the media. Wheat straw and rice husks followed by barley straw + rice husks supplemented with crushed sunflower seeds cultivated with *P. cornucopiae* gave the highest concentration of pleurotin after harvesting the fruit bodies, while corn cobs gave the lowest concentration of pleurotin in the treatment of fungus *Pleurotus eryngii* var. *ferulae* after fruiting. The reasons for this may be due to the fact that this protein source (crushed sunflower seeds) is suitable for the biosynthesis of pleurotin, this fact agreed with study of [29]. wheat straw also may contain fibers, starches , nitrogenous and phenolic compounds that help excrete pleurotin in the medium as indicated by [26].

[30,31] indicated the C:N ratio (28 30% carbon to 1% nitrogen) , one of the necessary conditions for the oyster mushroom growth and increasing its biological activity. As for the rice husks, it is a material rich in lignin more than cellulose or hemi-cellulose. This explains the superiority of the growth of oyster mushrooms in this medium and giving the highest rate of pleurotin concentration compared to other media, or it may be attributed to the high phenolic content in the rice husks and barley straw, as [25,26] recording the highest percentage of total phenols was found in the rice husks, followed by barley straw, as it reached 18.31 and 15.03 mg / g extract, respectively.

Palm fibers showed efficiency in raising pleurotin in the MEB medium, and this may be due to the fact that the quality of the fibers stimulates the production of pleurotin through enzymes that decompose the lignin such as peroxidase, polyphenol oxidase, and laccase, with the liberation of single or double phenolic rings, which are the basic units for the biosynthesis of pleurotin, and this is consistent with [16] who mentioned that some types of fiber stimulate the production of pleurotin. Due to the properties of pleurotin produced from oyster mushrooms against the fungal and bacterial growth, we believe that the fungus produces pleurotin as a defensive substance in which oyster mushrooms compete with the rest of the microorganisms that compete with it for nutrients and growth in the same medium. Through the results, pleurotin showed compatibility with pesticides Othilotop, Sabithane and Switht , this explains the existence of a synergistic effect between pleurotin and these fungicides, while it is noted that there is an antagonistic effect with Folio Gold, perhaps due to interactions in the chemical composition of both the Folio Gold and pleurotin, which leads to a decrease in the activities of each.

Conclusion

The oyster mushrooms tested in this study showed high efficiency in producing pleurotin after fruiting, so the isolate *P. cornucopiae* among five of *Pleurotus* spp. showed its superiority by producing the highest pleurotin concentration after fruiting in all tested media. The solid medium of wheat straw and rice husks supplemented with crushed flower grains was the best for pleurotin production while the liquid medium of Malt extract broth supplemented with the palm fronds powder was the best for pleurotin production. pleurotin was more compatible with fungicides Othilotop, Sabithane and Switht for inhibition of the pathogenic fungi, *Rhizoctonia solani* and *Alternaria alternate.*

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Increase Farm Profits by Electronic Control of the Harvester

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Abstract. A practical experiment was conducted on harvest loss and its impact on the farmer in terms of the amount of grain that can be lost during harvesting, and the amount of money lost as a result of this loss. The farmer obtains it from increasing harvesting efficiency, which will give less losses and better grain marketing. It was conducted in the province of Kirkuk, Hawija district, Al-Abbasi district, using a NEW HOLLAND TC54 BIZON alopecia model 2013 in the experiment, with a monitoring system consisting of a number of cameras connected with a humidity sensor and using the RCBD random sectors system. In the research, two main factors were used: the speed of the fan for cleaning A with three rates (1100, 1200, 1300) rpm, and combined with the ground speed of the harvester B, which had three speeds (3, 4.5, 6) km / h. For this experiment, it was chosen to study the following characteristics, which are the amount of grain that is scattered behind the harvester without supervision (Y1), then calculating the same trait by monitoring (Y2), the percentage of loss compared to the normal harvest (Y3), the harvest by electronic monitoring (Y4), and calculating the money lost by the farmer (Y5) Also, the money recovered was calculated by reducing losses, which are considered profits for the farmer (Y6) . The results showed that changing the rotational speed of the cleaning fan by obtaining the first speed is the best possible for the studied results, except in the case of the percentage of profits that was the largest at A3, by increasing the income of the farmer by 40 thousand dinars, by controlling the work of the harvester through the system, as for the ground speed, most of the characteristics increased. With the increase in the forward speed of the harvester, this was due to confusion in the work of the harvester by increasing the quantities entering from the harvest and the lack of good control over its work .The best results through the difference between working without a system and with the system was at the third fan speed with the third ground speed and it is considered the best achievement because we need to work at high speeds to reduce time and expenses and thus get the best performance.

Keywords. Electronic Control, Harvesting, Crop.

1. Introduction

Appropriate and controlled harvesting operations give the farmer an increase in his revenues, which were less as a result of the losses, the loss resulting from the scattering of grain behind the harvester, the main reason for which is the lack of control of the harvester driver over all its units, especially by changing the working conditions of the terrain and conditions of the crop, and this requires continuous organizations from Before the driver, whenever he changes the location of a field, especially now, there are many varieties for each type of crop, and each variety needs a specific regulation, and one of

these regulations is the forward speed, as [1], proved the extent of the direct and immediate impact of the ground speed by searching the season for the factors affecting the loss quantitative by speed selection, One of the main factors is the choice of three ground speeds, which are (3, 4, 4.5) km / h, and among the known things that they confirmed is that the high speeds will give a greater loss of grain, which came because of the high vibrations of the parts of the harvester, and the lack of angular movement of the picking fingers with the forward speed of the harvester with An increase in the amount of harvest that crosses into the harvester, Thad causes an irregularity in the work of the harvester and increases losses. Another factor that they highlighted is the height of the stems, which changes from the To another, whether in the same field or in another field, where the driver may keep the harvest at one height, and leads to agglomeration of the harvest inside and leads to more losses. As for [2], with his experience in measuring the quantitative loss of wheat by changing the type of variety for the wheat crop and its impact by proving that by using two varieties of wheat with changing both the cylinder speed and changing the concave openings, he noticed that the loss in soft wheat was 5.78% Compared to the loss of coarse wheat, which gave a total loss rate of 3.5%. The main reason for the great loss is the improper calibration of the harvester, which is what is required each driver to re-calibrate when moving from one field to another to change the type.

The feeding rates and fan regulation have an apparent effect on work efficiency in terms of losses and grain cleaning, and we notice this through the development of the harvesting machines that he developed [3], in order to reduce the weight of the developed harvester. Most of the machines that come from some countries may not be suitable for our regions, and this is one of the problems facing the import of agricultural machines. After the development procedure, the difference was noticed by increasing the feeding rates with a change in the fan speed and wind direction. The low feeding rate led to an increase in the damage to the grain, which was due to an increase in the collision rates between the grain and the dias cylinder, and this led to the breaking of the grain. This damage was reduced by increasing the input materials, but to a certain extent. When it is increased more, it leads to overlapping and cramming of the internal working parts. Also, cleaning changes with increasing speed and gives a cleaner crop, but it may lead to throwing the grains back.

The wind blown by the fans to clean the harvest before collecting it in the harvester tank and this is one of the most important things that the driver must consider. The appropriate calibration for it is the speed of rotation and the direction of the wind, which is what [4], tended to through their design of a cleaning fan for the harvester by increasing the number of blades on the fan to 8 blades, controlling the wind direction to increase the moment of inertia in order to control the cleaning operations that they considered important thing, When adding feathers to the fan, he was able to reduce the speed of the fan's rotation while giving a larger amount of air and momentum to the air, which gives a better cleaning quality. From the original design, with a reduction in the amount of losses in fallen grains and pushed back, and thus achieving a clear economic profit for farmers by increasing the extracted and accumulated quantities. They attributed the reason to the sufficient time and the required amount of air with the appropriate direction of the air direction.

[5], explained that the use of modern technologies in agricultural operations leads to full benefit from crop production, and it helps reduce effort and reduce losses to which farmers are exposed by using modern monitoring methods and from a distance using monitoring and sensitivity sensors with a geographical positioning system (GPS) by giving readings with mapping of the target sites, which facilitate the researcher’s work and facilitate finding the most loss areas with identifying the most causes, Through these readings it will give a clearer picture of the harvesting operations and with the use of the (DGPS) application, precision agriculture by dividing the field data with the sensors used to benefit from them for the season . All these technologies come in order to improve agricultural production by developing equipment and monitoring it while using it, while giving a clearer picture of the agricultural operations that will come for the next season. This is what he studied [6], in order to determine the benefit in using the positioning system for agricultural operations [7]

The use of information related to agricultural operations , gives the person in charge of agricultural operations the best expectations and helps him reduce the effort required of him to manage the agricultural machine, which is what [8], found in their search for giving a harmonious relationship for harvesting operations that are used in regulating the speed of the harvester units, especially the speed

The front of the harvester with the speed of rotation of the cylinder with the speed of the fan, and all these procedures were done using an electrical circuit linked to sensors to use the integrated management system, where these operations gave profitable results despite the presence of many obstacles such as moisture of the crop with the conditions , of the crop Through all that we have taken, he confirmed that the electronic management of agricultural operations had the benefit of giving the best picture and the best possible production, which makes the farmer happy by increasing the money obtained from his farm.
He also praised [9], that farmers are affected by the losses they are exposed to and the extent of the need to continue working in order to reduce these losses while giving them an increase in the incoming funds and all these losses that the farmer is exposed to from the beginning and after the agricultural operations, especially when delaying operations Marketing that, when immediately controlled, will increase the farmer's financial input , And that's agree with [10]

2. Materials and Methods

The experiment was carried out in Kirkuk, Hawija district, for the season 2022, for the spring corn crop, the harvester used (NEW HOLLAND TC54 BIZON model 2013) , and the RCBD random block design was used in the experiment. The experiment was conducted twice, the first was by using the harvester without using any electronic system, and then it was conducted again with the use of an electronic system consisting of the following:

- Cameras of the type of fences with a resolution o 4 external 5MP cameras
- 3.6mm fixed lens aperture
- Night photography at a distance of 20m
- Works on AHD, TVI, CVI, CVBS systems
- DVR: 4-channel DVR recorder
- Receives a volume of up to 6TB
- Contains 4 Audio IN ports
- Complete viewing by phone or calculator through the Internet
- Supports AHD, TVI, CVI, CVBS, IPC systems
- Toslink and Charger: It contains 4 cables with a length of 20m
- Joker DC 12V truck.

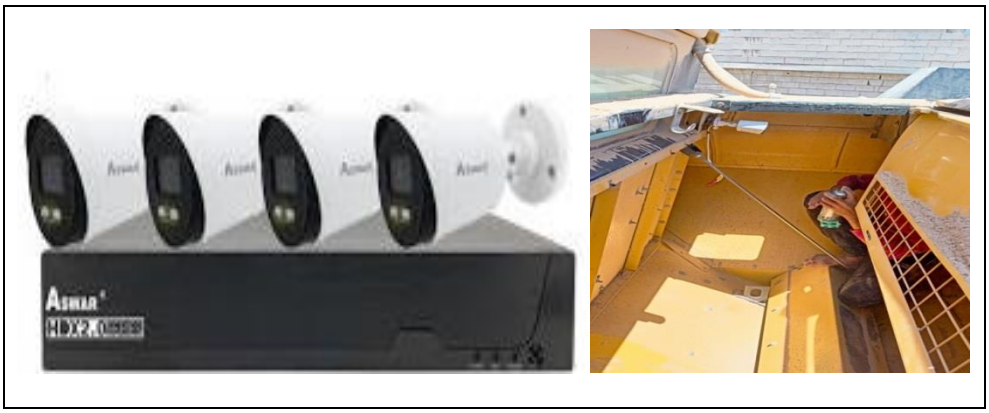

Figure 1. Cameras of the type of fences with a resolution o 4 external 5MP cameras.

The yield moisture during harvesting was 20%. Data were taken from the field using a frame measuring 1m x 1m to collect the scattered grains in the field, and then their weight was measured using an accurate balance of 0.01 gm. Two factors were used in the experiment:
First: The Fan Rotation Speed (A) was in Three Levels (1100, 1200 and 1300) rpm.
Second:. ground speed of the harvester (B) which had three speeds (3,4.5,6) km/ h.
The characteristics studied are:

- (Y1) the amount of grain that is scattered behind the harvester without supervision.

- (Y2) calculating the same trait by monitoring.
- (Y3) the percentage of loss compared to the normal harvest.
- (Y4) the harvest by electronic monitoring.
- (Y5) calculating the money lost by the farmer Also.
- (Y6) Farm profits by minimizing losses.

3. Results and Discussion

3.1. First: The Effect of rpm Fan Rotation Speed

Through our observation of Table No. (1), the increase in the rotational speed of the fan led to undesirable results, by increasing the percentage by about (8%) from the first speed to the third . However ,this percentage was reduced to reach about (3%), which is the same case for the adjective (Y3, Y4), and this is considered the main criterion for the harvester, although the percentages were within the permissible limit, but we were able to reduce the total percentage of losses from (9%) to (5%), which led to reducing losses in order to increase the income for the farmer, and this was among the topics confirmed by [11].

Table 1. The effect of fan speed on the studied characteristics.

A rpm	Studied traits					
	Y1	Y2	Y3	Y4	Y5	Y6
A1	5.36 c	4.46 c	5.28 c	3.02 c	26.29 c	8.07 c
A2	10.12 b	9.11 a	10.06 b	6.45 b	50.16 b	17.24 b
A3	13.85 a	7.72 b	14.05 a	8.39 a	70.20 a	30.75 a

3.2. Second: The Effect of the Forward Speed Km / hr

The second table showed the effect of the forward speed of the harvester, which had a varying effect between the studied traits. The best results for most traits were at the first speed. This was due to giving the harvester and its units full freedom during work by reducing the harvest entering it. But when using the electronic system, the percentages for each trait were reduced by (4%) to (6%), especially for high speeds, while giving the best profits to the farms, by increasing the profit up to 27 thousand dinars per dunam, and these results are the result of the successful management of the harvester, with changing the regulations during work and without stop and at the same time give the appropriate profit to the owner of the harvester by reducing work time and wasted time in organizing operations [12]. [13]

Table 2. The effect of forward speed on the studied characteristics.

B	Studied traits					
Km/ hr	Y1	Y2	Y3	Y4	Y5	Y6
B1	7.85 b	6.04 b	7.47 c	4.87 b	37.20 c	12.16 b
B2	10.78 b	8.62 a	9.87 b	6.24 a	49.27 b	16.98 b
B3	10.70 a	6.62 b	12.05 a	6.77 a	60.18 a	26.92 a

3.3. Bilateral Overlap in the Studied Traits

The best that was obtained in relation to Table No. (3) was for trait (Y1) at the slow forward speed because the harvester introduced a small amount of crop that led to its complete separation with a low fan speed, and this reduced the scattering of grain behind the harvester while giving very high results, changing matters when increasing the speed of the two workers together or when increasing the speed of one of them, and this , is gives an inappropriate combination of the working units. And through the electronic system, we were able to control the work of the harvester and its regulations, and the harvester worked in the field, and this led to a reduction in high-speed losses to about (9%), which is a very impressive reduction percentage at (Y2), which is the same as what we got in my class (Y3, Y4) and all This had the clearest effect on increasing the profit of the farms, and all of these changes were

by using the appropriate change of harvester calibrations at the right time, which was confirmed by [14]. And that say [15]. [16]

Table 3. Shows the effect of the bilateral interference between fan speed A rpm and forward speed B km/hr on the studied characteristics.

A	B	Studied traits					
		Y1	**Y2**	**Y3**	**Y4**	**Y5**	**Y6**
	B1	3.28 e	2.64 g	3.18 e	2.18 d	15.77 f	3.72 d
A1	B2	7.85 d	6.20 ef	6.33 e	3.66 d	31.61 e	11.05 b-d
	B3	4.96 e	4.53 F	6.32 e	3.32 d	31.50 e	9.44 c-d
	B1	8.78 cd	8.24 cd	8.84 d	5.66 c	44.05 d	14.61 b-d
A2	B2	9.73 b-d	10.31 a	9.88 cd	6.64 c	49.27 cd	15.05 b-d
	B3	11.85 b	8.80 ab	11.46 bc	7.05 b	57.16 cb	22.05 bc
	B1	11.50 bc	7.25 cd	10.37 c	6.75 c	51.77 cd	18.16 bc
A3	B2	14.76 a	9.36 a	13.41 b	8.41 b	66.94 b	24.83 b
	B3	15.28 a	6.55 de	18.37 a	10.02 a	91.68 a	49.27 a

Conclusion

The use of some modern technologies such as sensors and cameras in agricultural operations, including harvesters, gives the best results, by giving the best work to the driver with less fatigue, and at the same time the farmer benefits from them by reducing the types of losses that he obtains during harvesting, which leads to reducing his losses while giving him more profit.

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Response *Pistacia weinmannifolia* to Plant Growth Regulators *in Vitro*

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Abstract. This study was carried out in the laboratory of plant tissue culture department of biology, college of science university of Al-Qadisiyah , included several experiments on the response of *pistacia weinmannifolia* to some growth regulators and the effect of auxins and cytokines in vitro under light and dark condition. Grow them on MS medium with added concentration of growth regulators and they are incubated In the growth room under the influence of the light and dark factor at temperature of 25 ^{0}C and the results are taken every week from 45 days and after completing the cultivation the results gave that the pest combination in light for the growing apex (BA,IBA). And (KIN,IPA) where they gave a percentage of (100) As for the single node the best combination was (BA,2,4-D) which amounted to (92.58), In the case of dark. The best combination for the growing apex (KIN,IBA),(KIN,IPA) gave a percentage of (100), As for the single node the best combination (BA, 2,4-D) was 80% As for the formation of callus, the best synthesis In the state of light Is (Kin, 2.4-D), where It gave the highest percentage of 92.59%. In the case of dark, the best combination was (BA,NAA), where It gave the highest percentage 85.71%

Keywords. *Pistacia weinmannifolia*, Plant growth regulators, Cytokines, Auxins, *in Vitro.*

1. Introduction

Pistacia weinmannifolia belongs to the Anacardiaceae family and Evergreen shrub mainly distributed south western china including Yunnan, Sichuan guangxi ,guizhou [1], Owing to it's elegant profile antibacterial properties and capacity to Repel flies and mosquitoes [2]. Pistacia has become a popular ornamental plant, used for miniascapes , fencing and so on. Furthermore the leaves of Pistacia are rich in phenolic compounds, among which two novel gallotannins, pistafolin A and pistafolin B, are identified.In the present investigation the antioxidant effeciency of pistafolin A and pistafolin B in preventing lipid, protein and DNA from reactive oxygen species mediated damage was studied.Also the leaves of this plant are used in Chinese Folk medicine to treat dysentery, enteritis influenza, tranmatic bleeding headache and lung cancer [3,4]. Pistacia is used as a herbal medicine in China [5], and it's major metabolites possess biological activities such as inhitory activities against histamine release [6,7], in aprevious study,it was confirmed that the anti-inflammatory activities of Pistacia root extract PWER in PMA tumour necrosis factor a stimulated airway epithelial cells and pulmonary inflammatory response induced by cigarette smoke and lipopolysaccharide [8]. The technique of plant tissue culture or cultivation outside living body is the process of isolating a cell , tissue or organ from

the mother plant under sterile artificial nutrient mediums and then incubating the controlled conditions of temperature light and humidity in order to develop towards the desired goal. Either to acomplete plant [9,10], or the spread of callus.which is used in plant propagation tissue. Tissue culture depends on the cells' own ability to divide (totipotency), which means that each cell of the plant cells has the ability to divide, grow, and produce complete plants identical to the mother plant, as appropriate conditions are provided for it such as heat, light, and humidity, as well as nutrients and growth regulators [11]. That it is possible to grow different plant parts in the food media and obtain complete plants from them and can be used to improve productivity and the production of medicines and medical drugs to obtain seedlings free from disease infections, for example rapid propagation, which is one of the most important applications on tissue culture and also can benefit from tissue culture in the growth of some Plants that grow in hard-to-reach areas. They can be used for the purpose of producing secondary metabolite compounds under controlled sterile conditions [12]. Growth regulators are known as plant hormones prepared synthetically and used to regulate plant growth. And that growth regulators have the most important role in the success of micro-reproduction. Plant growth is the outcome of the growth of all its cells, tissues and organs. Among the most important growth regulators are auxins and cytokines, and they are frequently used in media, where auxins work on cell enlargement and start rooting, while cytokines work on cell division and the start of vegetative branching. Using them together leads to stimulating callus growth, and the ratio of auxins to cytokines can be manipulated in the laboratory to affect the growth of callus and vegetative branches [13,14].

2. Material and Method

This experiment was carried out in the plant tissue culture lab. Department of Biology, College of Science,Al-Qadisiyah University during October 2022 to January 2023 to study the influence of growth regulators (IAA,IBA,IPA,NAA,2,4D), Cytokinin (BA,Kin) and light conditions on some traits of callus and vegetative The purpose of this study is to investigate the response of *Pistacia weinmannifolia* plant to growth regulators and callus formation in vitro using tissue culture technique.

Figure 1. Plant growth chamber.

2.1. Prepare MS Media

Murashige and Skoog media was prepared by adding 1.11mg/L of MS media for 250ml from distilled water and 7.5mg/l sucrose. After that added the plant growth hormone (lAA,NAA,lPA,lBA2,4-D) at concentration of 0.2ml.After that the media placed on a hot plate magnetic stirrer and turned the movement only to mix the media and set the PH at 5.7 by use (NaOH) or (HCl) Then added 1.75mg/l from agar and left at a temperature of 70 until it become transparent.Then distribution 6ml for each sterile culture jar (35ml capacity) and sterilized the culture media by Autoclave at 120^0C under pressure 1bar in for 20 min. The medium was left at room temperature until hardened and ready for cultivation. After that it's incubated in the growth room at temperature 25^0C Fig 1.

2.2. Sterilization Explant

p. weinmannifolia was collected from the mother plant (the growing apex and lateral buds) by one node 0.5_1.0 cm. We removed the leaves from the plant branches After that we washed the plant to get rid of the dust and left under tap water for 30 minutes. Explant was sterilized by ethyl alcohol 70% for 1 min. and sodium hypochlorite fas(10%) for period of 25 min. with continuous shaking. After that the plant parts washed with distilled water 3 times to remove the minor substances and ethel alcohol from the plant parts. This sample were incubated at 25 ^{0}C and illumination about 1000 lux for 8 hr dark and 16 hr light. The tools such as blades, tweezer, scissors were sterilized using an electric oven at 160 for 2 hrs. As well as ethel 95% was used in the sterilization of the blades and tweezers. Bunsen burner was used after each use Tubes required for cultivation are sterilized using autoclaves at 120 ^{0}C.

3. Result and Discussion

3.1. The Effect of the Concentration of Sterilization Solution (Sodium Hypochlorite) on Emergence Explant of Pistacia Weinmannifolia After 5,10,15 Days of Cultivation in MS Medium in Vitro

The results of Table (1) indicate the efficiency of sodium hypochlorite at certain concentrations in sterilizing plant parts, and the presence of a significant effect of sodium hypochlorite concentrations on the percentage of growing plants. Sodium hypochlorite dilution ratio of 12% gave the highest response rate of 53.76 and at dilation ratio of 10% it gave the lowest response rate of 39.74. As for the effect of number of days, the results showed a significant effect of the number of days in increasing the percentage of plantings as it gave a response rate for growing implants after 15 days at a rate of 67.72 and the lowest response rate after 5 days, when the percentage reached 23.07. The results of statistical analysis showed that the overlap between the variables (concentration the number of days) had an effect on the average number of days. The same table showed a significant difference between the treatments . 12% dilution ratio gave the highest rate of 90.32, while the 10% Dilution ratio gave the lowest rate of 15.38. Fig 2.

Table 1. The effect of the concentration of sterilization solution (sodium hypochlorite) on emergence explant of *Pistacia weinmannifolia* after 5,10,15 days of cultivation in MS medium *in vitro.*

Dilution ratio	Days			Mean Dilution
	5 days	10 days	15 days	
8%	34.48	44.82	55.17	44.82
10%	15.38	46.15	57.69	39.74
12%	19.35	51.61	90.32	53.76
Mean days	23.07	47.52	67.72	
RLSD 0.05	Mean Dilution 1.8 Mean days 1.32 Intervention 1.76			

3.2. The Effect of the Concentration of Sterilization Solution (Sodium Hypochlorite) on Explants Contamination of Pistacia Weinmannifolia After 5,10,15 Days of Cultivation in MS Medium in Vitro

The results of table (2) indicate that the greater the number of days, the greater the percentage of concentration of explant as the highest of contamination at 10% dilution was 7.68. While the lowest percentage of contamination was at 8% dilution as the percentage reached 4.18. The results showed that the number of days had an effect on the contamination of explant ,as the highest contamination percentage reached 9.36 after 15 days of explant, and the lowest contamination percentage was 2.97 after 5 days. The results of the statistical analysis showed that the overlap the variables has a significant effect between the treatments and the 10% dilution rate gave the highest pollution rate of 11.53 and the lowest pollution rate at 8% dilution amounted to 6.89.

Table 2. The effect of the concentration of sterilization solution (sodium hypochlorite) on explants contamination of *Pistacia weinmannifolia* after 5,10,15 days of cultivation in MS medium *in vitro*.

Dilution ratio	Days			Mean Dilution
	5 days	10 days	15 days	
8%	2.22	3.44	6.89	4.18
10%	3.84	7.69	11.53	7.68
12%	3.22	6.45	9.67	6.44
Mean Days	2.97	5.86	9.36	
RLSD 0.05	Mean Dilution 0.32 Mean Days 0.45 Intervention 1.31			

It is clear from the results in tables (1 and 2), the effectiveness of sodium hypochlorite in reducing contamination of explant through surface sterilization without side effects at limited concentrations because it contains Hypochlorous Acid (HOCl) as a strong oxidant and easily removed.

3.3. The Effect of Plant Growth Regulators on Emergence Explants of Pistacia Weinmannifolia After 30 Days of Cultivation in Medium (MS) for the Plant Incubated in Light 16 Hours and 8 Hours Dark

The results of table (3) indicate that the Cytokinins have a significant effect on the response rate of the growing explants to the light as the best percentage of the growing apex is BA where the percentage reached 61.64%. As for the single node BA is also better as it gave the highest rate of 60.16%. As for the effect of Auxins IPA gave highest rate of 70% for the growing apex. As for the single node 2,4D gave the highest rate of 80.77%. As for the effect of intervention the best combination were (BA,IBA) and (kin, IPA) where both gave the highest rate of 100 for the growing apex. As for single node (BA,2,4-D) it gave the highest rate of 92.58% Fig 3.

Table 3. The effect of plant growth regulators on emergence explants of *Pistacia weinmannifolia* after 30 days of cultivation in medium (MS) for the plant incubated in light 16 hours and 8 hours dark.

Explant type	Auxine type	Cytokinin type		Mean Auxin
		BA	KIN	
Apical meristem	IAA	75.00	33.00	54.00
	IPA	40.00	100	70.00
	IBA	100	33.12	66.56
	NAA	33.22	50.00	41.61
	2,4-D	60.00	50.14	55.07
Mean Cytokinin		61.64	53.25	
RLSD $_{0.05}$		Auxine 1.84	Cytokinin 1.25	Intervention 2.13
Single node	IAA	92.30	46.15	69.22
	IPA	25.00	60.00	42.50
	IBA	26.66	55.55	41.10
	NAA	64.28	44.44	54.36
	2,4-D	92.58	68.96	80.77
Mean Cytokinin		60.16	55.02	
RLSD $_{0.05}$		Auxine 1.01	Cytokinin 1.39	Intervention 2.08

3.4. The Effect of Plant Growth Regulators on Emergence Explants of Pistacia Weinmannifolia After 30 Days of Cultivation in Medium (MS) for the Plant Incubated in the Dark

The results of table 4 indicate that the Cytokinins have effect on the response of emergence explants in the dark. The best ratio of the Cytokinins Kin as it gave the highest percentage of 55.20% for the growing apex. As for the single node BA is better because it gave the highest percentage of 52.76%. As for the effect of Auxins IBA gave the highest percentage of 75.06% for the growing apex , while for a single node 2,4-D gave the highest rate of 60%. As for the Intervention effect the best combination were (Kin,IPA) and (Kin, IBA) both of which gave the highest rate of 100 for the growing apex . As for the single node (BA, 2,4-D) it gave the highest rate 80%.

Table 4. The effect of plant growth regulators on emergence explants of *Pistacia weinmannifolia* after 30 days of cultivation in medium (MS) for the plant incubated in the dark.

Explant type	Auxine type	Cytokinin type		Mean Auxin
		BA	KIN	
Apical meristem	IAA	50.00	12.00	31.00
	IPA	33.00	100	66.50
	IBA	50.12	100	75.06
	NAA	50.00	14.00	32.00
	2,4-D	33.33	50.00	41.66
Mean Cytokinins		43.29	55..20	
RLSD $_{0.05}$		Auxine 1.91	Cytokinin 1.34	Intervention 2.86
Single node	IAA	60.00	40.12	50.06
	IPA	26.66	71.42	49.04
	IBA	40.00	60.00	50.00
	NAA	57.14	40.00	48.57
	2,4-D	80.00	40.00	60.00
Mean Cytokinins		52.76	50.30	
RLSD $_{0.05}$		Auxine 1.64	Cytokinin 1.06	Intervention 2.21

3.5. The Effect of Plant Growth Regulators on Callus Formation of Pistacia Weinmannifolia After 45 Days of Cultivation in MS Medium for the Plant Incubated in Light 16 Hours and 8 Hours in Dark

The results of Table (5) indicate that Cytokinin have effects on callus formation in the light, as the best ratio for Cytokinin (BA), which gave the highest percentage of 69.49%. As for the effect of Auxins, (2,4-D) gave the highest percentage of 85.87% As for the effect of Intervention between them, the best combination is (kin, 2,4-D) gave the highest rate of 92.59% Fig 4.

Table 5. The effect of plant growth regulators on the percentage of callus formation after 45 days of cultivation in MS medium for the plant incubated in light 16 hours and 8 hours in dark.

Auxine type	Cytokinin type		Mean Auxine
	KIN	BA	
IAA	62.50	80.00	71.25
IPA	11.76	25.00	18.38
IBA	33.33	80.00	56.66
NAA	66.66	83.33	74.99
2,4D	92.59	79.16	85.87
Mean Cytokinin	53.36	89.49	
RLSD 0.05	Auxine 1.32	Cytokinin 1.29	Intervention 1.97

3.6. The Effect of Plant Growth Regulators on Callus Formation of Pistacia Weinmannifolia After 45 Days for Plants Grown on MS Medium in the Dark

The results of Table (6) indicate that Cytokinin have effects on callus formation in the dark, as the best ratio of Cytokinin BA, which gave the highest percentage of 51.80%. As for the effect of Auxins, 2,4-D gave the highest percentage of 80.%. As for the effect of Intervention between them, the best combination was (BA, NAA), which gave the highest percentage of 85.71%.

Table 6. The effect of plant growth regulators on callus formation of *Pistacia weinmannifolia* after 45 days for plants grown on MS medium in the dark.

Auxine type	Cytokinin type		Mean Auxine
	KIN	BA	
IAA	25.00	33.00	29.00
IPA	22.22	22.00	22.11
IBA	16.00	33.33	24.66
NAA	60.00	85.71	72.85

Auxine type	Cytokinin type		Mean Auxine
	KIN	BA	
2,4-D	75.00	85.00	80.00
Mean Cytokinin	39.64	51.80	
RLSD 0.05	Auxine 1.53	Cytokinin 1.05	Intervention 1.89

Figure 2. The effect of the concentration of sterilization solution (sodium hypochlorite) on emergency explant of *Pistacia weinmannifolia* after 5, 10, 15 days of cultivation in MS medium in vitro.

Figure 3. The effect of growth regulators on emergency explants of *Pistacia weinmannifolia* after 30 days.

Figure 4. The effect of plant growth regulators on callus formation of *Pistacia weinmannifolia* after 45 days.

The results in tables (3,4,5 and 6) showed a clear percentage of emergence explants and callus formation due to the effect of growth regulators, where cytokinin to the medium work to disrupt the hormonal balance in the apical meristem that rich auxin at the production site [15,6], as well as the role of cytokinin in the mechanism of attracting and accumulating metabolites at the sites of buds. The side effects and stimulation of the transfer of nutrients, other growth materials, and minerals are

necessary to start the growth of the vegetative system, as well as a direct catalyst for the construction of protein, RNA, and chlorophyll [17,18]. The mechanism of the mutual influence of cytokinin and auxin in the doubling and elongation of branches resulting from the cultivation of ganglia can be explained on the basis the cytokinin stimulates cell division and increases the number and size of cells [19], so it is necessary for its presence with auxin for the purpose of activating the process of cell division and elongation of cells through the role of auxin in increasing the softness of cell walls and increase their permeability, which increases cell expansion and large size [20,21].

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