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Effect of Water Deficiency in Different Stages of Potato (*Solanum tuberosum* L.) Growth

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Abstract: The experiment was conducted to assess the sensitivity of potato yield to different irrigation levels at different growth stages. Irrigation levels were determined as percentages (WI 100% as readily available water to the crop (RAW)), and for the rest of the treatments (WII 75%, WIII 50%), as they were applied separately to all four stages of crop growth i.e., vegetative (SI), tuber initiation (SII), tuber bulking SIII, and tuber maturation (SIV). The design of complete random sectors was adopted to perform the experiments. The results revealed that all the studied parameters: plant height (cm), vegetation plant weight (g), number of tubers per plant, tuber weight (g), tuber yield (ton/ha), and crop water productivity (kg/m^3) varied among irrigation water levels at different stages of growth. It was found that the two stages, SII and SIII in potato crops, were more sensitive to deficit irrigation compared to other stages. According to the obtained results, in the case of water abundance conditions, the treatment WII SI can be applied to obtain the highest water crop productivity. In conditions of water scarcity, it becomes necessary to apply the treatment WIII SIII to obtain the highest crop water productivity.

العجز المائي عند مراحل نمو مختلفة للبطاطس (*Solanum tuberosum* L)

الكلمات المفتاحية : البطاطس، العجز المائي، الإجهاد المائي، كفاءة استخدام المياه، الانتاجية المائية المحصولية.

المستخلص : تهدف هذه الدراسة إلى تحديد تأثير العجز المائي عند مراحل النمو المختلفة علي إنتاجية محصول البطاطس. تم تحديد معاملات الري كنسب مئوية (WI 100%) كمياه متاحة للمحصول بسهولة (RAW)، ولبقية المعاملات (WII 75%، WII 50%)، حيث تم تطبيقها علي جميع مراحل نمو المحصول الأربعة: النمو الخضري (SI) تكوين الدرنات(SII) ، ملء الدرنات (SIII) و نضج الدرنات (SIV) كل على حدا، وضمن تصميم القطاعات العشوائية الكاملة. أظهرت نتائج التجربة الحقلية المعاملات المختلفة التي تم دراستها والتي اشتملت على ارتفاع النبات(سم)، وزن المجموع الخضري (جم)، عدد الدرنات لكل نبات، وزن الدرنات (جم)، الانتاجية (طن/هكتار)، والانتاجية المائية المحصولية (كجم/م^3) كان بينها اختلافات متباينة بين مستويات مياه الري في مراحل النمو المختلفة. وجد أن المرحلتين الثانية (SII) و الثالثة (SII) كانتا الأكثر حساسية للري الناقص مقارنة بالمراحل الأخرى. وفقا للنتائج المتحصل عليها، وفي حال ظروف الوفرة المائية، فإنه بالإمكان تطبيق المعاملة WII SI للحصول على أعلى انتاجية مائية. أما عند ظروف ندرة المياه، يصبح من الضروري تطبيق المعاملة WIII SIII للحصول على أعلى انتاجية مائية.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the fourth crop produced worldwide after rice, wheat, and corn, with a total global production of 365 million tons in 2012 and a cultivated area of 18.6 million hectares (FAO, 2014). There has been a dramatic increase in potato production and demand in Asia, Africa, and Latin America, where the yield rose from less than 30 million tons in the early 1960s to more than 381 million tons in 2014 (PotatoPro, 2014). Potato is a water-stress-sensitive

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crop. Potato plants are more productive and produce higher tuber quality when watered precisely using soil water tension than if they are under or over irrigated (Hashem et al., 2016; Rosen et al., 2014). Also, potato is sensitive to deficit irrigation throughout various growth stages, especially in tuber formation up to harvest (Badr et al., 2022; Bahramloo & Nasseri, 2009; Shock, 2004; Thornton, 2002) found the sensitivity of potatoes to water stress was more remarkable when water was applied at individual growth stages than at different severities of water stress. Potato development is differentiated into four growth stages, namely: sprout growth, tuber initiation, tuber bulking, and tuber maturation (Struik & Wiersema, 1999). The period of these growth stages is controlled by the environmental and management factors related to locations as well as the plant variety (Doorenbos & Pruitt, 1977; Warsito & Van de Fliert, 2006). All these developmental stages can be identified on early, mid, and late cultivars and are like those suggested by (Van Loon, 1981).

The vegetative growth stage begins from the planting date and extends to the stolon's formation. The duration of the vegetative growth stage ranges from 30 to 70 days and depends on varieties, cultural practices, and environmental conditions (Patil & Sundaresha, 2016). The tuber initiation stage takes around 20 to 30 days (Cowan, 1986). According to (Kang et al., 2004), the tuberization stage begins when the stolon tip starts to swell and the tuber begins to develop, which lasts from 10 to 14 days. Although additional stolons may continue to form during later stages of plant growth, marketable tubers are formed during this stage. The tuber bulking growth stage extends from the time when tubers are about one-half inch in diameter to the beginning of canopy senescence with a duration of 60 to 120 days. This wide variation is deeply dependent upon variety, nutrient availability, and environmental conditions (Kang et al., 2004). As indicated by the International Potato Center (CIP, 1984), potato tuber swelling has a period of 60 to more than 120 days, contingent upon the length of the developing season and presence of pathogens. Tuber maturation begins with canopy senescence. The growth of the tuber shows a lower rate during maturation than during the tuber bulking stage (Ojala et al., 1990). The tuber bulking period is the time between tuber initiation and duration of foliage, while tuber bulking growth can be depicted by the slant of a straight bend, with the expansion in tuber mass after some time (Ojala et al., 1990). Tubers' weight may reach up to 300 g each. Generally, they differ in size and shape (FAO, 2008).

Water stress during the vegetative growth stage reduces leaf area, root expansion, and plant height and delays canopy development. There is an agreement among agricultural specialists including (Braue et al., 1983; Ojala et al., 1990 ; Kempen,2012), upon the fact that water stress during the maturation stage would be accompanied by a decline in photosynthesis rate, regression in the tuber development rate, and the vine dieback. The impact of water deficit through the different growth stages should be known before implementing a stress irrigation program. It is necessary to know crop yield responses to water stress (Kirda & Kanber, 1999) at different stages.

This study aimed to assess the sensitivity of potato yield to different irrigation levels at different growth stages in the Jordan Valley**.**

MATERIALS AND METHODS

Experimental site and weather conditions: The study was carried out at the Agricultural Experimental Station of Jordan University in the Jordan Valley. The Station is located at 32^{o}50' N and 35^{o}34'E. The altitude is 370 m below sea level. Climate is warm in winter and hot in summer. The average minimum temperature is 18.5℃ and maximum temperature is 30℃, with annual rainfall ranging from 100 to 150 mm.

Land preparation and soil data collectio: The field was tilled by disc plough to approximately 30 cm depth. The field was divided into plots and then it was completely flooded by water. For determining the required physical and chemical characteristics, three composite samples (2kg) were collected from each soil layer (0-20, 20-40, and 40 – 60 cm). According to (Ababsa, 2013),

the soil of the experimental site was classified as Hyperthermic, Typic Torriorfluents. Different soil properties were measured including field capacity and permanent wilting point which was determined using ceramic plate (Cassel & Nielsen, 1986). The bulk density of soil was determined by the core method with a soil volume of 63 cm^3 (Blake & Hartge, 1986). The soil texture was performed using the pipette method (Gee et al., 1986). Calcium carbonate in soil was estimated by the sodium hydroxide method (Nelson, 1982). Electrical conductivity and pH of the soil were determined in (1:1) soil extract (McLean, 1982). Phosphorus was determined according to (Olsen, 1982). The Kjeldahl digestion-distillation method was used to estimate the nitrogen content in the soil (Bremner & Mulvaney, 1982). Potassium was extracted by acetic acid and measured by a flame photometer system (Knudsen et al., 1983). Some physical and chemical properties of the soil are presented for the different soil layers in Table (1).

Table (1). Some physical and chemical properties of the soil.

Soil depth (cm)	0 -20	20-40	40-60
Texture	Sandy clay loam	Sandy clay loam	Sandy clay loam
Bulk density (gm/cm^3)	1.43	1.47	1.42
FC (cm^3/cm^3)	0.293	0.285	0.298
PWP (cm^3/cm^3)	0.139	0.129	0.136
EC (dS/m)	0.467	0.627	0.473
pH	7.4	8.01	8.1
$CaCO_3$ (%)	24.9	25.6	24.7
Total N (%)	0.45	0.51	0.49
P (ppm)	60.7	40.6	42
K (ppm)	62.4	56.1	52.1

Growth stages: The length of the growing stages of potatoes depends on planting date, soil temperature, climate, location, and other environmental factors (Doorenbos & Pruitt, 1977; Lisinska & Leszczynski, 1989) In this study, the potato (*Solanum tuberosum* L.) cultivar˝Spunta˜was grown under three different irrigation treatments, which were initiated at each growth stage to the end of the growing season. The length of potato growing stages was determined based on visual observations and recognizing their characteristics as mentioned by Johnson (2008) as follows:

Stage 1(SI): Vegetative growth. It extended up to 48 days after planting tubers (DAP). Stolon's formation started when plant height was17 cm;

Stage 2 (SII): Tuber initiation. It took 20 days after stage I and depended on stolon's development. The swelling of tubers reached to less than one inch.

Stage 3 (SIII): Tuber bulking. It took 33 days after stage II and tubers were about one-half of its final of this stage.

Stage 4 (SIV): Tuber maturation. It took 18 days after stage III depending on the chlorophyll percentage in leaves. Table (2) depicts the cauterization of potato growth stages.

Experimental Design: The experiment consisted of 12 treatments that resulted from the combination of three different irrigation treatments (factor 1) and four growth stages (factor 2) with three replications using a factorial arrangement in Randomized Complete Block Design (RCBD). The size of each unit plot was 8 m in length with a width of 5 m; plants were spaced at 0.40 m within rows and 0.80 m between rows. Each plot contained 100 plants. Plots were separated by 2 m from each other within the plot and 3 m between replicates. Tubers were manually planted on Dec 20^{th} 2015 with a density of 3.125 plant/m^2.

Table (2). Characterization of plant growth stages

Growth stages of potato	Stages(S)	Date	Number of days
Planting		20/12/2015	0
Vegetative	SI	05/02/2016	48
Tuber initiation	SII	25/02/2016	20
Tuber bulking	SIII	29/03/2016	33
Tuber maturation	SIV	16/04/2016	18
Total number of days	119		

Plant harvesting: The potato tubers were harvested on 16th April 2016 and the harvested plot`s size was 11.52 m^2 (three rows at the center of each plot (4.80 m× 2.40 m). At the physiological maturity stage, vegetation weight, tubers weight, tuber number, and potato yield per treatment and hectare were measured. Mean measured values were taken per plant for tubers' weight and plant height.

Estimation of irrigation supply: The three irrigation treatments were 100, 75, and 50% of the readily available water (RAW). They were irrigated on the same dates with different durations for each treatment when the measured volumetric soil water content of 100% reaches the critical value. The management allowable depletion was taken as 40% of total available water. Soil water content was monitored in each plot using calibrated time domain reflectometer (TDR). The soil water content was measured for each 0.2 m soil layer before and after each irrigation using an access tube with a diameter of 5.5 cm holes. A drip irrigation system was used with one irrigation source line and drippers spaced 0.4 m with an average discharge of 4 Lh^{-1}.

The distribution efficiency of emitters was evaluated by the discharge of the three emitters per line and showed a flow difference of 9.5%. Detecting irrigation treatments were considered at the following four developmental stages. Table (3) depict the irrigation treatments and their details.

Fertilizer application: All treatments were supplied with the recommended amount of fertilizer (255 kg N (ammonium sulfate 21%) ha^{-1}, phosphorus 70 kg of P ha^{-1} (20- 20 -20-Trace elements), and 132 kg of K ha^{-1} (12 -12 -36 +total elements) through the irrigation water in all treatments. According to (Demelash, 2013), fertilizer requirements are 80 to 120 kg ha^{-1} N, 50 to 80 kg ha^{-1} P and 120 to 160 kg ha^{-1} K, depending on soil analysis and irrigated crop.

Table (3). Irrigation treatments and their details

Irrigation treatment	Details
WI	100% of RAW: Irrigation amount for all stages (SI, SII, SIII and SIV).
SIWII	75% of RAW at SI and 100% of RAW during SII, SIII and SIV.
SIWIII	50% of RAW at SI and 100% of RAW during SII, SIII and SIV.
SIIWII	75% of RAW at SII and 100% of RAW during SI, SIII and SIV.
SIIWIII	50% of RAW at SII and 100% % of RAW during SI, SIII and SIV.
SIIIWII	75% of RAW at SIII and 100% of RAW during SI, SII and SIV.
SIIIWIII	50% of RAW at SIII and 100% of RAW during, SI, SII and SIV
SIVWII	75% of RAW at SIV and 100% of RAW during SI, SII and SIII.
SIVWIII	50% of RAW at SIV and 100% % of RAW during SI, SII and SIII.

Water management: Irrigation water was managed in relation to the soil moisture level. The first treatment (100%) was effected by applying all the moisture extracted from effective root zoon (ERZ) when the depletion percentage (Dp) reaches 40% of RAW. The volumetric soil water content was performed by using TDR for the different layers of ERZ at different depths i.e., 0-20, 20-40, and 40-60 cm. The second treatment was planned for 75 % of full irrigation, and the third level by the application of 50% only. Through this low-discharge emitters irrigation system there will be no runoff and consequently,

this term had been neglected in the water balance equation. Deep percolation has been minimized through the low rate of application and checked by the measurement of the moisture content below the root zone (60-90 cm) before planting and after harvest. Irrigation scheduling was based on the soil moisture balance measurement, including the amount of each application and timing (Gheysari et al., 2009).

$$NRD = \sum_{i=0}^{n}(\theta_{FC} - \theta_{sb})D_i \quad (1)$$

Where, NRD refers to the net irrigation depth (mm) which was applied at irrigation time. θ_{FC} refers to soil water content at field capacity (cm^3cm^{-3}), D_i refers to the thickness of each soil layer (mm), θ_{sb} refers to soil water content before irrigation time (cm^3cm^{-3}) at 40% of RAW.

Therefore, consumed water (CW) was calculated by applying the water balance equation to the (ERZ) 60 cm.

$$ET = I - \left(R + D_p\right) \pm \Delta\theta \quad (2)$$

Where, ET refers to evapotranspiration (mm), I refers to the amount of applied irrigation water (mm), R refers to the surface runoff (mm) which is negligible, Dp refers to the deep percolation (mm), and Δθ refers to the change in the soil water storage prior to planting and after harvesting of the soil profile above 60 cm depth (mm) (Watson & Burnett, 1995). (Pereira & Shock, 2006) showed that ET of potato crop in duration 120 to 150 days was 500 to 700 mm, depending on atmosphere conditions.

Crop water productivity and water use efficiency: The concepts of water use efficiency (WUE) and water productivity of crops are important indicators for evaluating the water consumption of crops (Ekhmaj & Almuntaser, 2016). The term water use efficiency is used in different ways by agronomists and physiologists depending upon the emphasis that one wishes to place on certain aspects of the problem. Agronomists define this term as crop yield per unit of water use (Sinclair et al., 1984). The total water lost by evapotranspiration and transpiration is often used for this purpose. As 99% of the water consumed by crops in the field is transpired from crop leaves and evaporated from the soil surface, water use efficiency in effect, is the reciprocal of evapotranspiration. Physiologists define the WUE concept in terms of the process of photosynthesis, expressing it in milligrams of CO_2 per gram of water (Kramer, 2012).

Water productivity (WP) can be defined as the ratio of the economic yield of a crop to the total water supply diverted to irrigate the crop (Alghariani, 2006) Both diverted and consumed water include the sum of the total water flow in addition to the net flow and water depleted by the crop (Molden, 1997; Molden & Sakthivadivel, 1999). Thus, in agricultural systems, and in terms of water consumed by the crop, crop water productivity is considered to be a measure of the output of that system. However, to reach the goal of determining the crop water productivity for the whole agricultural system, this must be done in time and space (Gichuki et al., 2006).

In this study, two indicators were adopted to assess water consumption, which are the water productivity of crops at the field level (WP) or the production yield per unit volume of irrigation water and water use efficiency (WUE). The following equations show the mathematical formulas for these indicators:

$$WP = \frac{Y}{I} \quad (3)$$

$$WUE = \frac{Y}{ET_c} \quad (4)$$

Where Y refers to potato yield for each treatment (kg/ha), (I) is applied irrigation water for each treatment (m^3/ha) and ET_c is potato evapotranspiration (m^3/ha)

Statistical analysis: Data collected from the field experiment was statistically analyzed using SAS program version 8. Analysis of variance (ANOVA) was used to estimate the significance of irrigation treatments and stages. Means showed significant differences were separated by the least significant difference (LSD) test at $P<0.05$.

RESULTS AND DISCUSSIONS

Water balance of the root zone: Initial, final, and the change of soil water depths within the root zone of potatoes during entire growth stages and water treatments are depicted in figure (1). It was found that the initial soil water depths (at the beginning of the experiment) were uniform within the root zone at 80 mm. The final soil water depths (at harvesting the crop) were between 58 mm (during the SIV with WIII) and 78 mm (during the SIII with WI). The changes in soil water depth were determined as a difference between initial soil water depths and final water depths. The changes in soil water depths were between 2 mm (during the SIII with WI) and 23 mm (during the SI with WI). The changes in soil water depths were used to determine the water balance of potato cultivation.

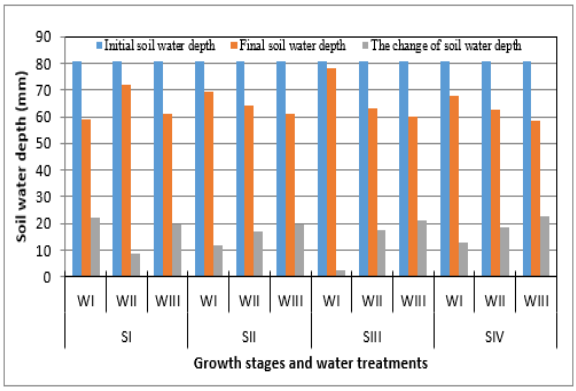

Figure (1). Potato crop evapotranspiration for the different treatments.

Table (4) shows the water balance for the different treatments. Essentially, the water supply was based on irrigation and soil water content since there was no precipitation occurring throughout the whole four growth stages. The deep percolation was noted to be in small amounts, and the runoff was ignored as no runoff was noticed. The maximum and the minimum of evapotranspiration were 711 and 561 mm, for SIWI and SIII WIII, respectively.

Deficient irrigation treatments led to a reduction in evapotranspiration at different rates according to the plant growth stages. It showed differences among irrigation treatments, as well. The maximum decrease in evapotranspiration, 8.1% and 18.8%, were noticed during tuber bulking stage in the second and third irrigation treatments, respectively. The reason for this, is that the quantities of irrigation water added to the full irrigation treatment were higher than the incomplete irrigation treatments, which led to an increase in the processes of transpiration by the plant and evaporation from the soil surface. In addition, it was also found that the highest evapotranspiration was at the vegetative growth stage, the tuber emergence stage, and tuber formation. However, these results are similar to the literature data (Onder et al., 2005; Sadiq, 2013).

Plant height: Results, as shown in Figure (2), explained that irrigation water during all growth stages has no significant effect between water levels, while there was a significant effect between stages under the significant level of (0.05). The average plant height tends to increase under the irrigation treatment of WII at the SI, at which the highest height of 51 cm was recorded. The deficit irrigation treatment of WIII gave the least average height at the SIV, recording 41 cm tall, and did not show any difference in all treatments of water at SII and SIII. It might be due to the fact that the crop encountered favorable soil moisture conditions, which enhanced the availability of nutrients essentially required for the enlargement and elongation of plant cells. However, as it was indicated by (Zrust, 1995), that plant height was initially water sensitive for plant height, with a 20% reduction rate for full irrigation treatment, which was consistent, as well, with (Kang et al., 2004; Kashyap & Panda, 2003).

Table: (4). Water balance for the different treatments.

Stage	Treatment	ET (mm)	I (mm)	P (mm)	R (mm) (mm)	Dp (mm)	$\pm\Delta$ θ(mm)
SI	WI	702	692	0	0	3	13
	WII	677	670	0	0	3	9
	WIII	662	648	0	0	6	20
SII	WI	701	692	0	0	3	12
	WII	665	651	0	0	3	17
	WIII	624	609	0	0	5	20
SIII	WI	691	692	0	0	3	2
	WII	635	619	0	0	2	18
	WIII	561	545	0	0	5	21
SIV	WI	702	692	0	0	3	13
	WII	681	665	0	0	2	18
	WIII	654	637	0	0	5	23

Vegetation plant weight: The results showed the significant effect of irrigation treatments on vegetation plant weight at growth stages of SII, SIII, and SIV. On the other, irrigation treatment in SI has no significant difference in the average weight of vegetation plants which reached 607, 600, and 595g, respectively, in irrigation treatment WII, WI, and WIII, as indicated in figure (3). SII showed no significant difference between WII and WIII, showing that the lowest weights of plants of 400 and 382 g were respectively related to irrigation treatments of WII and WIII.

Number of tubers per plant: The effect of deficit irrigation treatments on the average number of tubers is different during all growth stages (Figure 4). While the number of tubers showed a significant effect at SII, it was obvious that the greatest number of tubers was recorded at SI WII with 10 tubers per plant. At the SII WIII stage, the average number of tubers was six because the deficit irrigation met the critical stage of tubers formation showing a clear negative effect on both physiological activity and produced metabolites.

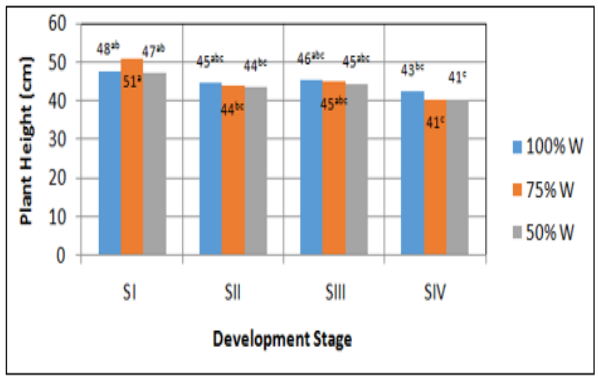

Figure (2). Effect of deficit irrigation treatments on the final heights of plant. LSD (P<0.05).

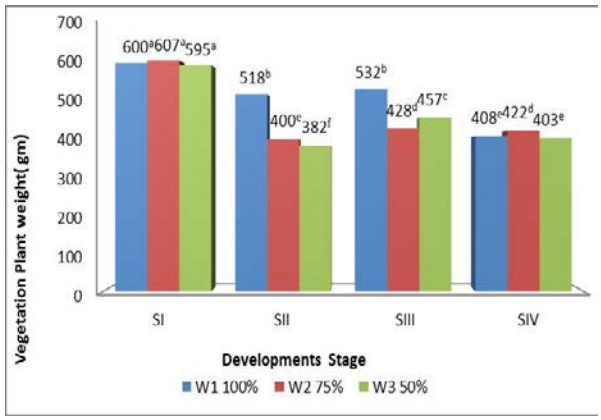

Figure (3). Effect of deficit irrigation on plant weight at growth stages. LSD (P<0.05).

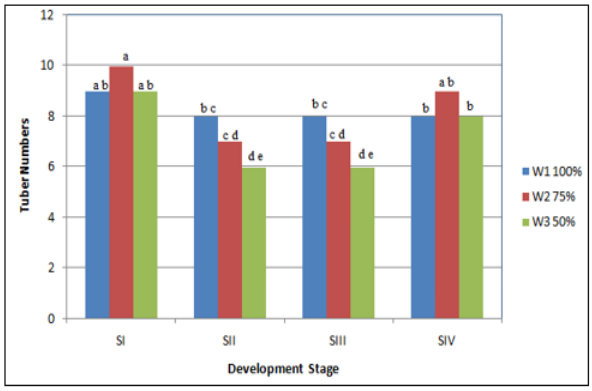

Figure (4). Effect of deficit irrigation treatments on the average tubers number per plant. LSD (P<0.05).

As indicated by (Zrust, 1995), that plant height was initially water sensitive for plant height, with a 20% reduction rate for full irrigation treatment, which was consistent with (Kang et al., 2004; Kashyap & Panda, 2003).

Tubers weight: The effect of water treatments and interaction between (water levels and growth stages) on the weight of tubers was insignificant except at growth stages of SII and SIII.

Figure (5) showed that the deficit irrigation treatment WII has a clear effect on tuber weight during both SI and SIV stages (1573 and 1432 g, respectively). The least average tubers weight of the deficit irrigation treatment under the irrigation level of WIII was 1105 g in SII of the growth and 1163 g in the SIII stage. The shortage in irrigation water during the tubers' formation and cell development stages tends to reduce tubers' growth. (Chang, 1968) also indicated that the moisture-sensitive stages of potatoes were from stolonization to the beginning of tuberization. So, any deficit in irrigation, if accompanied with high temperature, will break the dormancy of the recently formed tubers which will start to grow up in soil, and if the soil moisture increases, tubers will give a secondary small growth causing the low weight of tubers. These results agreed with (Bailey & Groves, 1992; Fabeiro et al., 2001; Sadiq, 2013).

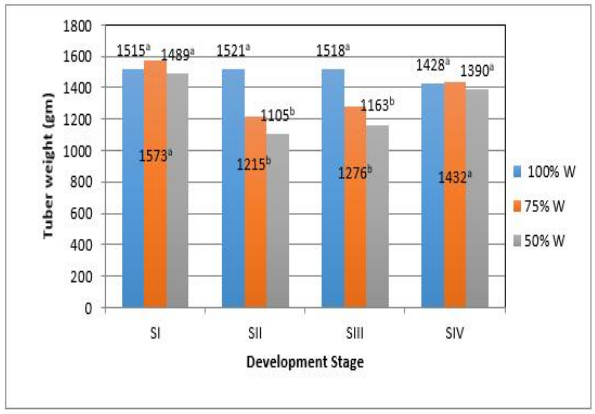

Figure (5). Effect of deficit irrigation treatment on average tubers weight per plant at various growth stages. LSD (P<0.05).

Tubers yield: The potato yield of the deficit irrigation treatments is shown in Figure (6). There was no significant difference between deficit irrigation treatments except for the SII growth stage, which had a significant difference. The interaction between deficit irrigation and growth stages was seen in SII. SIII has less effect than SII in all treatments of deficit irrigation. While the effect of treatments of deficit irrigation at the same stage was insignificant, the treatment 75% (WII) at SI gave more yield than treatment 100% (WI), while WI produced fewer yields. The lowest yield was found in treatment WIII with SII, and the highest yield was in treatment WII with SI. Other researchers as (Fabeiro et al., 2001), reported that 597 mm irrigation water was required to obtain a tuber yield of 45.18 t ha^{-1}. In another study, it was resulting that deficit irrigation should be avoided during tubers formation and at the middle of the maturity stage of potatoes to reach acceptable quality and quantity productivity (Kiziloglu et al., 2006). Water deficit has decreased the evapotranspiration and tuber yield of potatoes according to (Kiziloglu et al., 2006). These results of deficit irrigation showed that deficit irrigation had significant impact on yield. The amount of irrigation water was reduced as yield significantly decreased.

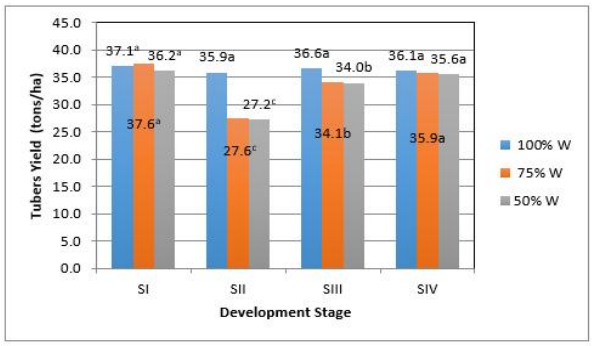

Figure (6). Tubers yield as affected by deficit irrigation treatment at different growth stages LSD (P<0.05).

Crop Water Consumption: Table (5) presents the results of water productivity (WP) and water use efficiency (WUE) for the different irrigation levels. The results showed that the best treatment in WP was recorded at deficit irrigation of WIII in SIII producing 6.231 kg/m^3, while the lowest yield value of 4.238 kg/m^3 was related to SII under deficit irrigation of WII, whereas the treatment WI at stages, SI, SII, SIII, and SIV gave 5.353, 5.187, 5.291 and 5.220 kg/m^3 respectively. Although the yield was 37589 kg ha-1 in the treatment of the irrigation deficit of WII at stage SI, its water productivity was 5.6 kg/m^3. (Rashidi & Gholami, 2008) illustrated that WP of potato in Iran ranged from 1.92 to 5.25 kg m^{-3}.Deficit irrigation had an effect on the yield and yield components at different levels of irrigation. Deficit irrigation had significant impact on yield and yield components at SII, which were obtained from levels WIII and WII which have the lowest. These results agreed with the research conducted by (Onder et al., 2005). Many irrigation studies indicated that a reduction in yield is a cause of deficit irrigation at different growth stages of the potato (Hassan et al., 2002).

The results of this study generally agree with the observation that an increase in water level 100% irrigation level leads to decreased WP (Erdem et al., 2006; Norwood, 2000; Shani & Dudley, 2001). The results of water use efficiency (kg/m^3) calculated from equation (4), showed that the maximum water use efficiency was 6.05 kg/m^3 at stage III and under irrigation treatment of WIII. On the other hand, the lowest value of WUE 4.15 (kg/m^3) was noted at stage II under irrigation treatment of WIII. WUE follow the same trend as WP. Stage II had the best values as compared with other stages. Like WP, the best values of WUE were also noted in WIII. The comparison between the values of the water productivity of crops and the efficiency of water use, both in units (kg/m^3), shows the loss of small quantities of water that the crop does not benefit from, which indicates the high efficiency of the irrigation systems used and the adoption of guided agricultural practices.However, by examining table (5), obtaining the largest possible crop yield regardless of the amounts of water added can be archived by adopting the WIISI treatment. Such intervention can be applied in cases of water abundance. On the other hand, regarding water scarcity, it is advised to apply the WIII SIII treatment to obtain the highest crop water productivity.

Table (5). Water productivity and water use efficiency for different stages and irrigation treatments.

Stages	Irrigation (level irrigation)	Crop Yield (kg/ ha)	Applied irrigation amount (m^3/ha)	$\frac{ET}{mm}$	$\frac{WP}{kg/m3}$	$\frac{WUE}{kg/m3}$
SI	WI	37061	6923	702	5.35	5.28
	WII	37589	6703	677	5.6	5.55
	WIII	36150	6484	662	5.57	5.46
SII	WI	35910	6923	701	5.18	5.12
	WII	27576	6507	665	4.23	4.15
	WIII	27227	6087	624	4.47	4.36
SIII	WI	36632	6923	691	5.29	5.3
	WII	34055	6191	635	5.5	5.36
	WIII	33966	5451	561	6.23	6.05
SVI	WI	36141	6923	702	5.22	5.15
	WII	35890	6646	681	5.4	5.27
	WIII	35586	6366	654	5.59	5.44

CONCLUSION

The results of this study found that the two stages SII (tuber initiation) and SIII (tuber bulking) in potato crop were the highest sensitivity to deficit irrigation compared to other stages. Thus, to obtain the best yield and yield components (number of tubers per plant, tuber weight, and plant weight of potato), Irrigation should be scheduled carefully during tuber formation. Based on the results obtained it can be recommended to avoid any reduction in the amount of irrigation water at the stage of tubers formation in order to obtain the highest tuber yield and better economic gains. Many similar studies must be conducted in different regions of Jordan since the soil and climate are different from one site to another.

Duality of interest: The authors declare that they have no duality of interest associated with this manuscript.

Author contributions: Taleb did the field study and collected data, also contributed of analysis, Suleiman and Abu-Rayyan designed the research framework and performed the analytic calculations. Both Ekhmaj and Benzaghta contributed to the final version of the manuscript. Suleiman and Abu-Rayyan supervised the project.

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Some Extensions and Generalizations of Kümmer's Third Summation Theorem

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Abstract: The motive of this research paper is to obtain explicit forms of certain extensions and generalizations of Kümmer's third summation theorem, which have not previously appeared in the literature, by using the summation theorem given by Rakha and Rathie (2011). The results derived in this paper are interesting and may be beneficial.

بعض الامتدادات والتعميمات لنظرية التجميع الثالثة لكومر

الكلمات المفتاحية : الدوال الفوق هندسية؛
نظرية التجميع الثالثة لكومر؛
نظريات التجميع الفوق هندسية.

المستخلص : دوافع هذا البحث هي الحصول على أشكال صريحة من بعض الامتدادات والتعميمات لنظرية (كومار) Kümmer الثالثة للتجميع، باستخدام نظرية التجميع التي قدمها سنة (2011) (رخا) Rakha و(راثي) Rathie والتي لم تظهر في مؤلفات سابقة. النتائج المستمدة من هذا البحث مثيرة للاهتمام وقد تكون مفيدة.

INTRODUCTION and PRELIMINARIES

The theory of hypergeometric functions is rapidly developing with a large number of applications in the real world. The major development was given (although Euler and Pfaff had found many important results) by Gauss and Kümmer on the series ${}_2F_1$ and ${}_1F_1$ respectively and the other higher order hypergeometric functions such as ${}_2F_1$ and ${}_3F_2$ presented by (Clausen, 1828; Goursat, 1883), respectively.

- A natural generalization of the Gaussian hypergeometric series ${}_2F_1[\alpha, \beta; \gamma; z]$ is accomplished by introducing any arbitrary number of numerator and denominator parameters. Thus, the resulting series:

$$ {}_pF_q\left[\begin{matrix}(\alpha_p);\\ \\ (\beta_p);\end{matrix} \quad z\right] = {}_pF_q\left[\begin{matrix}\alpha_1,\alpha_2,\ldots\alpha_p;\\ \\ \beta_1,\beta_2,\ldots\beta_p;\end{matrix} \quad z\right] $$

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$$= \sum_{n=0}^{\infty} \frac{\prod_{j=1}^{p} (\alpha_j)_n \; z^n}{\prod_{j=1}^{q} (\beta_j)_n \; n!}, \qquad (1.1)$$

is known as the generalized hypergeometric series, or simply, the generalized hypergeometric function. Here p and q are positive integers or zero, and we assume that the variable z, the numerator parameters $\alpha_1, \alpha_2, \ldots \alpha_p$ and the denominator parameters $\beta_1, \beta_2, \ldots \beta_q$ take on complex values, provided that

$$\beta_j \neq 0, -1, -2, \ldots \; ; \; j = 1, 2, \ldots q. \qquad (1.2)$$

Supposing that none of the numerator and denominator parameters is zero or a negative integer, we note that the ${}_pF_q$ series defined by equation (1.1):

(i) Convergence for $|z| < \infty$, if $p \leq q$;

(ii) Convergence for $|z| < 1$, if $p \leq q+1$;

(iii) Divergence for all $z, z \neq 1$, if $p > q+1$;

(iv) Convergence absolutely for $|z| = 1$, if $p = q+1$ and $\Re(w) > 0$;

(v) Converges conditionally for $|z| = 1$ $(z \neq 1)$ if $p = q+1$ and $-1 < \Re(w) \leq 0$;

(vi) Divergence for $|z| = 1$, if $p = q+1$ and $\Re(w) \leq -1$.

Where, by convention, a product over an empty set is interpreted as 1 and

$$w := \sum_{j=1}^{q} \beta_i - \sum_{j=1}^{p} \alpha_i. \qquad (1.3)$$

In this paper, we shall use the following standard notations:

$\mathbb{N} := \{1, 2, 3, \ldots\}$; $\mathbb{N}_0 := \mathbb{N} \cup \{0\}$;

$\mathbb{Z}_0^- := \mathbb{Z}^- \cup \{0\} = \{0, -1, -2, \ldots\}$. The symbols $\mathbb{C}$, $\mathbb{R}$, $\mathbb{N}$, $\mathbb{Z}$, $\mathbb{R}^+$ and $\mathbb{R}^-$ denote the sets of complex numbers, real numbers, natural numbers, integers, positive and negative real numbers, respectively.

- The Pochhammer symbol $(\alpha)_p$ [(Rainville, 1971), p.(22), Eq.(1), Q. N.(8) and Q. N.(9), see also (Srivastava & Manocha, 1984), p.(23), Eq.(22) and Eq.(23)] is defined by:

$$(\alpha)_p := \frac{\Gamma(\alpha + p)}{\Gamma(\alpha)}$$

$$= \begin{cases} 1 & ; (p = 0; \alpha \in \mathbb{C} \setminus \{0\}), \\ \alpha(\alpha+1)\ldots(\alpha+n-1) & ; (p = n \in \mathbb{N}; \alpha \in \mathbb{C}), \\ \dfrac{(-1)^k n!}{(n-k)!} & ; (\alpha = -n; p = k; n, k \in \mathbb{N}_0; 0 \leq k \leq n), \\ 0 & ; (\alpha = -n; p = k; n, k \in \mathbb{N}_0; k > n), \\ \dfrac{(-1)^k}{(1-\alpha)_k} & ; (p = -k; k \in \mathbb{N}; \alpha \in \mathbb{C} \setminus \mathbb{Z}), \end{cases}$$

it being understood conventionally that $(0)_0 = 1$ and assumed tacitly that the Gamma quotient exists (see, for details, [(Srivastava & Manocha, 1984), p.21 *et seq.*]). Here, we aim at the extensions and generalizations of Kümmer's third summation theorem involving the summation theorems given by Rakha and Rathie. Here, for the purpose of the present investigation, we would like to recall the following summation formula which is due to (Kummer, 1836).

- Kümmer's third summation theorem [(Kummer, 1836). p.134]:

$${}_2F_1\left[\begin{matrix} a, 1-a; \\ c; \end{matrix} \; \frac{1}{2}\right] = \frac{2^{1-c}\,\Gamma(c)\,\Gamma(1/2)}{\Gamma\left(\frac{c+a}{2}\right)\Gamma\left(\frac{1+c-a}{2}\right)} \qquad (1.4)$$

$$= \frac{\Gamma\left(\frac{c}{2}\right)\Gamma\left(\frac{c+1}{2}\right)}{\Gamma\left(\frac{c+a}{2}\right)\Gamma\left(\frac{1+c-a}{2}\right)} \qquad (1.5)$$

where $c \in \mathbb{C} \setminus \mathbb{Z}_0^-$.

In the literature, the above summation formula is also known as "Bailey's summation theorem".

- Summation theorem given by (Rakha & Rathie, 2011), p.828, Theorem (6)):

$$
{}_2F_1\left[\begin{matrix}\alpha, 1-\alpha+m; \\ \beta;\end{matrix}\ \frac{1}{2}\right]
= \frac{2^{1+m-\beta}\sqrt{\pi}\ \Gamma(a+m)\Gamma(\beta)}{\Gamma(\alpha)\Gamma\left(\frac{\beta-\alpha}{2}\right)\Gamma\left(\frac{\beta-\alpha+1}{2}\right)} \times
$$

$$
\times\sum_{n=0}^{m}\left\{(-1)^n\binom{m}{n}\frac{\Gamma\left(\frac{\beta-\alpha+n}{2}\right)}{\Gamma\left(\left(\frac{\beta+\alpha+n}{2}\right)-m\right)}\right\}, \tag{1.6}
$$

where $\alpha, \beta, \beta-\alpha, \alpha-m \in \mathbb{C} \setminus \mathbb{Z}_0^-$ and $m \in \mathbb{Z}_0$.

Motivated by the work collected in the beautiful monographs of (Andrews et al., 1999; Bailey, 1953; Carlson, 1977; Erdélyi et al., 1955; Prudnikov et al., 1986; Slater, 1966; Srivastava & Choi, 2011) and the papers of (Arora & Singh, 2008; Kim et al., 2010; Kim et al., 2013; Miller, 2005; Qureshi & Baboo, 2016; Qureshi & Khan, 2020), and others (Awad et al., 2021; Koepf et al., 2019), we are interested in giving some summation formulas for ${}_3F_2\left[1/2\right]$ in Section 2. In Section 3 and 4, we have given some summation formulas for ${}_4F_3\left[1/2\right]$ and ${}_5F_4\left[1/2\right]$ respectively. The detailed proof of summation formulas has been provided by using the summation theorem given by Rakha-Rathie and the series rearrangement technique.

Any values of the numerator and denominator parameters in sections 2, 3, and 4, leading to results which do not make sense are tacitly excluded.

2- Summation formulas for ${}_3F_2\left[1/2\right]$

Theorem 2.1. *The following summation theorem holds true*:

$$
{}_3F_2\left[\begin{matrix}a, 1-a+p, c+1; \\ b, c;\end{matrix}\ \frac{1}{2}\right]
= \frac{2^{1+p-b}\sqrt{\pi}\ \Gamma(b)}{\Gamma(a)\Gamma\left(\frac{b-a}{2}\right)\Gamma\left(\frac{b-a+1}{2}\right)} \times
$$

$$
\times\left[\Gamma(a-p)\sum_{r=0}^{p}\left\{(-1)^r\binom{p}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p}{2}\right)}\right\}+\right.
$$

$$
\left.+\frac{(1-a+p)\Gamma(a-p-1)}{c}\times\right.
$$

$$
\left.\times\sum_{r=0}^{p+2}\left\{(-1)^r\binom{p+2}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p-2}{2}\right)}\right\}\right], \tag{2.1}
$$

where $a, b, c, b-a, a-p \in \mathbb{C} \setminus \mathbb{Z}_0^-$ and $p \in \mathbb{Z}_0$.

Proof of Theorem (2.1): In order to establish the result, we proceed as follows:

$$
{}_3F_2\left[\begin{matrix}a, 1-a+p, c+1; \\ b, c;\end{matrix}\ \frac{1}{2}\right] =
$$

$$
= \sum_{r=0}^{\infty}\frac{(a)_r(1-a+p)_r(c+1)_r(1/2)^r}{(b)_r(c)_r r!}
$$

$$
= \sum_{r=0}^{\infty}\frac{(a)_r(1-a+p)_r(1/2)^r}{(b)_r r!}\left\{1+\frac{r}{c}\right\}
$$

$$
= \sum_{r=0}^{\infty}\frac{(a)_r(1-a+p)_r(1/2)^r}{(b)_r r!}+\frac{1}{c}\sum_{r=1}^{\infty}\frac{(a)_r(1-a+p)_r(1/2)^r}{(b)_r(r-1)!} \tag{2.2}
$$

Replacing r by $r+1$in the second term on the right hand side of the equation (2.2), we get:

$$
{}_3F_2\left[\begin{matrix} a,1-a+p,\, c+1; \\ b,\, c; \end{matrix}\ \begin{matrix} \\ \frac{1}{2} \end{matrix}\right]
$$

$$
=\sum_{r=0}^{\infty}\frac{(a)_r(1-a+p)_r(1/2)^r}{(b)_r\, r!}+\frac{1}{c}\sum_{r=0}^{\infty}\frac{(a)_{r+1}(1-a+p)_{r+1}(1/2)^{r+1}}{(b)_{r+1}\, r!}
$$

$$
=\sum_{r=0}^{\infty}\frac{(a)_r(1-a+p)_r(1/2)^r}{(b)_r\, r!}+
$$

$$
+\frac{a(1-a+p)}{2bc}\sum_{r=0}^{\infty}\frac{(1+a)_r(2-a+p)_r(1/2)^r}{(1+b)_r\, r!}
$$

$$
={}_2F_1\left[\begin{matrix} a,1-a+p\ ; \\ b; \end{matrix}\ \begin{matrix} \\ \frac{1}{2} \end{matrix}\right]+
$$

$$
+\frac{a(1-a+p)}{2bc}\,{}_2F_1\left[\begin{matrix} 1+a,\ 2-a+p\ ; \\ 1+b; \end{matrix}\ \begin{matrix} \\ \frac{1}{2} \end{matrix}\right]
$$

(2.3) Applying summation theorem (1.6) in equation (2.3) , we get

$$
{}_3F_2\left[\begin{matrix} a,1-a+p,\, c+1; \\ b,\, c; \end{matrix}\ \begin{matrix} \\ \frac{1}{2} \end{matrix}\right]=
$$

$$
=\frac{2^{1+p-b}\sqrt{\pi}\ \ \Gamma(b)\Gamma(a-p)}{\Gamma(a)\Gamma\left(\frac{b-a}{2}\right)\Gamma\left(\frac{b-a+1}{2}\right)}\times
$$

$$
\times\sum_{r=0}^{p}\left\{(-1)^r\binom{p}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\left(\frac{b+a+r}{2}\right)-p\right)}\right\}+
$$

$$
+\frac{2^{1+p-b}\,(1-a+p)\sqrt{\pi}\ \ \Gamma(b)\Gamma(a-p-1)}{c\Gamma(a)\Gamma\left(\frac{b-a}{2}\right)\Gamma\left(\frac{b-a+1}{2}\right)}\times
$$

$$
\times\sum_{r=0}^{p+2}\left\{(-1)^r\binom{p+2}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\left(\frac{b+a+r+2}{2}\right)-(p+2)\right)}\right\},
$$

(2.4) On simplifying further, we arrive at the result (2.1) .

Theorem 2.2. *The following summation theorem holds true*:

$$
{}_3F_2\left[\begin{matrix} a,1-a+p,\, c+2; \\ b,\, c; \end{matrix}\ \begin{matrix} \\ \frac{1}{2} \end{matrix}\right]
$$

$$
=\frac{2^{1+p-b}\sqrt{\pi}\ \ \Gamma(b)}{\Gamma(a)\Gamma\left(\frac{b-a}{2}\right)\Gamma\left(\frac{b-a+1}{2}\right)}\times
$$

$$
\times\left[\Gamma(a-p)\sum_{r=0}^{p}\left\{(-1)^r\binom{p}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p}{2}\right)}\right\}+\right.
$$

$$
+\frac{2(1-a+p)\Gamma(a-p-1)}{c}\times
$$

$$
\times\sum_{r=0}^{p+2}\left\{(-1)^r\binom{p+2}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p-2}{2}\right)}\right\}+
$$

$$
+\frac{(1-a+p)(2-a+p)\Gamma(a-p-2)}{c\,(c+1)}\times
$$

$$
\left.\times\sum_{r=0}^{p+4}\left\{(-1)^r\binom{p+4}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p-4}{2}\right)}\right\}\right],
$$

(2.5)

where $a, b, c, b-a, a-p \in \mathbb{C} \setminus \mathbb{Z}_0^-$ and $p \in \mathbb{N}_0$.

Theorem 2.3. *The following summation theorem holds true:*

$$
{}_3F_2\left[\begin{matrix} a, 1-a+p, c+3; \\ b,\ c; \end{matrix}\ \frac{1}{2}\right]
= \frac{2^{1+p-b}\sqrt{\pi}\ \Gamma(b)}{\Gamma(a)\Gamma\left(\frac{b-a}{2}\right)\Gamma\left(\frac{b-a+1}{2}\right)}\times
$$

$$
\times\left[\Gamma(a-p)\sum_{r=0}^{p}\left\{(-1)^r\binom{p}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p}{2}\right)}\right\}+\right.
$$

$$
+\frac{3(1-a+p)\Gamma(a-p-1)}{c}\times
$$

$$
\times\sum_{r=0}^{p+2}\left\{(-1)^r\binom{p+2}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p-2}{2}\right)}\right\}+
$$

$$
+\frac{3(1-a+p)(2-a+p)\Gamma(a-p-2)}{c(c+1)}\times
$$

$$
\times\sum_{r=0}^{p+4}\left\{(-1)^r\binom{p+4}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p-4}{2}\right)}\right\}+
$$

$$
+\frac{(1-a+p)(2-a+p)(3-a+p)\Gamma(a-p-3)}{c(c+1)(c+2)}\times
$$

$$
\left.\times\sum_{r=0}^{p+6}\left\{(-1)^r\binom{p+6}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p-6}{2}\right)}\right\}\right],
$$

(2.6)

where $a, b, c, b-a, a-p \in \mathbb{C} \setminus \mathbb{Z}_0^-$ and $p \in \mathbb{N}_0$.

Theorem 2.4. The following formula holds true:

$$
{}_3F_2\left[\begin{matrix} a, 1-a+p, c+4; \\ b, c; \end{matrix}\ \frac{1}{2}\right]
= \frac{2^{1+p-b}\sqrt{\pi}\ \Gamma(b)}{\Gamma(a)\Gamma\left(\frac{b-a}{2}\right)\Gamma\left(\frac{b-a+1}{2}\right)}\times
$$

$$
\times\left[\Gamma(a-p)\sum_{r=0}^{p}\left\{(-1)^r\binom{p}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p}{2}\right)}\right\}+\right.
$$

$$
+\frac{4(1-a+p)\Gamma(a-p-1)}{c}\times
$$

$$
\times\sum_{r=0}^{p+2}\left\{(-1)^r\binom{p+2}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p-2}{2}\right)}\right\}+
$$

$$
+\frac{6(1-a+p)(2-a+p)\Gamma(a-p-2)}{c(c+1)}\times
$$

$$
\times\sum_{r=0}^{p+4}\left\{(-1)^r\binom{p+4}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p-4}{2}\right)}\right\}+
$$

$$
+\frac{4(1-a+p)(2-a+p)(3-a+p)\Gamma(a-p-3)}{c(c+1)(c+2)}\times
$$

$$
\times\sum_{r=0}^{p+6}\left\{(-1)^r\binom{p+6}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p-6}{2}\right)}\right\}+
$$

$$
+\frac{(1-a+p)(2-a+p)(3-a+p)(4-a+p)\Gamma(a-p-4)}{c(c+1)(c+2)(c+3)}\times
$$

$$
\left.\times\sum_{r=0}^{p+8}\left\{(-1)^r\binom{p+8}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p-8}{2}\right)}\right\}\right], \quad (2.7)
$$

where $a, b, c, b-a, a-p \in \mathbb{C} \setminus \mathbb{Z}_0^-$ and $p \in \mathbb{N}_0$.

The proof of theorems (2.2)-(2.4), would run parallel to theorem (2.1) with the help of summation theorem (1.6) and the series rearrangement technique. The involved details are omitted.

3- Summation formulas for ${}_4F_3\left[\frac{1}{2}\right]$

Theorem 3.1. *The following summation theorem holds true*:

$$
{}_4F_3\left[\begin{matrix} a, 1-a+p, c+1, d+1; \\ b, c, d; \end{matrix}\ \frac{1}{2}\right]
= \frac{2^{1+p-b}\sqrt{\pi}\ \Gamma(b)}{\Gamma(a)\Gamma\left(\frac{b-a}{2}\right)\Gamma\left(\frac{b-a+1}{2}\right)} \times
$$

$$
\times\left[\Gamma(a-p)\sum_{r=0}^{p}\left\{(-1)^r\binom{p}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p}{2}\right)}\right\}+\right.
$$

$$
+\frac{(1-a+p)(1+c+d)\Gamma(a-p-1)}{cd}\times
$$

$$
\times\sum_{r=0}^{p+2}\left\{(-1)^r\binom{p+2}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p-2}{2}\right)}\right\}+
$$

$$
+\frac{(1-a+p)(2-a+p)\Gamma(a-p-2)}{cd}\times
$$

$$
\left.\times\sum_{r=0}^{p+4}\left\{(-1)^r\binom{p+4}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p-4}{2}\right)}\right\}\right], \tag{3.1}
$$

where $a, b, c, d, b-a, a-p \in \mathbb{C} \setminus \mathbb{Z}_0^-$ and $p \in \mathbb{N}_0$.

Proof of the Theorem (3.1):

In order to establish the result, we proceed as follows.

$$
{}_4F_3\left[\begin{matrix} a, 1-a+p, c+1, d+1; \\ b, c, d; \end{matrix}\ \frac{1}{2}\right] =
$$

$$
= \sum_{r=0}^{\infty}\frac{(a)_r(1-a+p)_r(c+1)_r(d+1)(1/2)^r}{(b)_r(c)_r(d)_r r!}
$$

$$
= \sum_{r=0}^{\infty}\frac{(a)_r(1-a+p)_r(1/2)^r}{(b)_r r!}\left\{1+\frac{(1+c+d)r}{cd}+\frac{r(r-1)}{cd}\right\}
$$

$$
= \sum_{r=0}^{\infty}\frac{(a)_r(1-a+p)_r(1/2)^r}{(b)_r r!}+\frac{(1+c+d)}{cd}\times
$$

$$
\times\sum_{r=1}^{\infty}\frac{(a)_r(1-a+p)_r(1/2)^r}{(b)_r(r-1)!}+
$$

$$
+\frac{1}{cd}\sum_{r=2}^{\infty}\frac{(a)_r(1-a+p)_r(1/2)^r}{(b)_r(r-2)!} \tag{3.2}
$$

Replacing r by $r+1$in the second term and r by $r+2$ in the third term on the right hand side of the equation (3.2), we get

$$
{}_4F_3\left[\begin{matrix} a, 1-a+p, c+1, d+1; \\ b, c, d; \end{matrix}\ \frac{1}{2}\right] =
$$

$$
= \sum_{r=0}^{\infty}\frac{(a)_r(1-a+p)_r(1/2)^r}{(b)_r r!}+\frac{(1+c+d)}{cd}\times
$$

$$
\times\sum_{r=0}^{\infty}\frac{(a)_{r+1}(1-a+p)_{r+1}(1/2)^{r+1}}{(b)_{r+1} r!}+
$$

$$
+\frac{1}{cd}\sum_{r=0}^{\infty}\frac{(a)_{r+2}(1-a+p)_{r+2}(1/2)^{r+2}}{(b)_{r+2} r!}
$$

$$
= \sum_{r=0}^{\infty}\frac{(a)_r(1-a+p)_r(1/2)^r}{(b)_r r!}+\frac{(1+c+d)a(1-a+p)}{2bcd}\times
$$

$$
\times\sum_{r=0}^{\infty}\frac{(1+a)_r(2-a+p)_r(1/2)^r}{(1+b)_r r!}+
$$

$$
+\frac{a(a+1)(1-a+p)(2-a+p)}{4b(b+1)cd}\times
$$

$$\times\sum_{r=0}^{\infty}\frac{(2+a)_r(3-a+p)_r(1/2)^r}{(2+b)_r\,r!}$$

$$={}_2F_1\left[\begin{matrix}a,1-a+p;\\ \\ b;\end{matrix}\ \begin{matrix}\\ \frac{1}{2}\\ \\ \end{matrix}\right]+\frac{(1+c+d)a(1-a+p)}{2bcd}\times$$

$$\times{}_2F_1\left[\begin{matrix}1+a,\ 2-a+p;\\ \\ 1+b;\end{matrix}\ \begin{matrix}\\ \frac{1}{2}\\ \\ \end{matrix}\right]+$$

$$+\frac{a(a+1)(1-a+p)(2-a+p)}{4b(b+1)cd}\times$$

$$\times{}_2F_1\left[\begin{matrix}2+a,\ 3-a+p;\\ \\ 2+b;\end{matrix}\ \begin{matrix}\\ \frac{1}{2}\\ \\ \end{matrix}\right]. \qquad (3.3)$$

Now applying the summation theorem (1.6) in the equation (3.3), we get

$${}_4F_3\left[\begin{matrix}a,1-a+p,c+1,d+1;\\ \\ b,c,d;\end{matrix}\ \begin{matrix}\\ \frac{1}{2}\\ \\ \end{matrix}\right]=$$

$$=\frac{2^{1+p-b}\sqrt{\pi}\ \Gamma(b)\Gamma(a-p)}{\Gamma(a)\Gamma\left(\frac{b-a}{2}\right)\Gamma\left(\frac{b-a+1}{2}\right)}\times$$

$$\times\sum_{r=0}^{p}\left\{(-1)^r\binom{p}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\left(\frac{b+a+r}{2}\right)-p\right)}\right\}+$$

$$+\frac{2^{1+p-b}(1-a+p)(1+c+d)\sqrt{\pi}\ \Gamma(b)\Gamma(a-p-1)}{c\,d\,\Gamma(a)\Gamma\left(\frac{b-a}{2}\right)\Gamma\left(\frac{b-a+1}{2}\right)}\times$$

$$\times\sum_{r=0}^{p+2}\left\{(-1)^r\binom{p+2}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\left(\frac{b+a+r+2}{2}\right)-(p+2)\right)}\right\}+$$

$$+\frac{2^{1+p-b}(1-a+p)(2-a+p)\sqrt{\pi}\,\Gamma(b)\Gamma(a-p-2)}{cd\,\Gamma(a)\Gamma\left(\frac{b-a}{2}\right)\Gamma\left(\frac{b-a+1}{2}\right)}\times$$

$$\times\sum_{r=0}^{p+4}\left\{(-1)^r\binom{p+4}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\left(\frac{b+a+r+4}{2}\right)-(p+4)\right)}\right\}.$$

(3.4) On simplifying further, we arrive at the result (3.1) .

Theorem 3.2. *The following summation theorem holds true*:

$${}_4F_3\left[\begin{matrix}a,1-a+p,c+1,d+2;\\ \\ b,c,d;\end{matrix}\ \begin{matrix}\\ \frac{1}{2}\\ \\ \end{matrix}\right]$$

$$=\frac{2^{1+p-b}\sqrt{\pi}\ \Gamma(b)}{\Gamma(a)\Gamma\left(\frac{b-a}{2}\right)\Gamma\left(\frac{b-a+1}{2}\right)}\times$$

$$\times\left[\Gamma(a-p)\sum_{r=0}^{p}\left\{(-1)^r\binom{p}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p}{2}\right)}\right\}+\right.$$

$$\left.+\frac{(1-a+p)(2+2c+d)\Gamma(a-p-1)}{cd}\times\right.$$

$$\left.\times\sum_{r=0}^{p+2}\left\{(-1)^r\binom{p+2}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p-2}{2}\right)}\right\}+\right.$$

$$\left.+\frac{(1-a+p)(2-a+p)(4+c+2d)\Gamma(a-p-2)}{cd(d+1)}\times\right.$$

$$\left.\times\sum_{r=0}^{p+4}\left\{(-1)^r\binom{p+4}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p-4}{2}\right)}\right\}+\right.$$

$$\left.+\frac{(1-a+p)(2-a+p)(3-a+p)\Gamma(a-p-3)}{cd(d+1)}\times\right.$$

$$\times \sum_{r=0}^{p+6}\left\{(-1)^r\binom{p+6}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p-6}{2}\right)}\right\}\Bigg], \tag{3.5}$$

where $a, b, c, d, b-a, a-p \in \mathbb{C} \setminus \mathbb{Z}_0^-$ and $p \in \mathbb{Z}_0$.

Theorem 3.3. *The following summation theorem holds true*:

$$ {}_4F_3\left[\begin{matrix} a, 1-a+p, c+2, d+2; \\ \\ b, c, d; \end{matrix} \middle| \begin{matrix} \\ \frac{1}{2} \\ \\ \end{matrix}\right]$$

$$= \frac{2^{1+p-b}\sqrt{\pi}\ \Gamma(b)}{\Gamma(a)\Gamma\left(\frac{b-a}{2}\right)\Gamma\left(\frac{b-a+1}{2}\right)} \times$$

$$\times\Bigg[\Gamma(a-p)\sum_{r=0}^{p}\left\{(-1)^r\binom{p}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p}{2}\right)}\right\}+$$

$$+\frac{(1-a+p)(4+2c+2d)\Gamma(a-p-1)}{cd}\times$$

$$\times\sum_{r=0}^{p+2}\left\{(-1)^r\binom{p+2}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p-2}{2}\right)}\right\}+$$

$$+\frac{(1-a+p)(2-a+p)\Gamma(a-p-2)}{c(c+1)}\times$$

$$\times\frac{(14+c^2+d^2+4cd+9c+9d)}{d(d+1)}\times$$

$$\times\sum_{r=0}^{p+4}\left\{(-1)^r\binom{p+4}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p-4}{2}\right)}\right\}+$$

$$+\frac{(1-a+p)(2-a+p)(3-a+p)}{c(c+1)}\times$$

$$\times\frac{(8+2c+2d)\Gamma(a-p-3)}{d(d+1)}\times$$

$$\times\sum_{r=0}^{p+6}\left\{(-1)^r\binom{p+6}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p-6}{2}\right)}\right\}+$$

$$+\frac{(1-a+p)(2-a+p)(3-a+p)}{c(c+1)}\times$$

$$\times\frac{(4-a+p)\Gamma(a-p-4)}{d(d+1)}\times$$

$$\times\sum_{r=0}^{p+8}\left\{(-1)^r\binom{p+8}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p-8}{2}\right)}\right\}\Bigg], \tag{3.6}$$

where $a, b, c, d, b-a, a-p \in \mathbb{C} \setminus \mathbb{Z}_0^-$ and $p \in \mathbb{Z}_0$.

Theorem 3.4. *The following summation theorem holds true*:

$$ {}_4F_3\left[\begin{matrix} a, 1-a+p, c+1, d+3; \\ \\ b, c, d; \end{matrix} \middle| \begin{matrix} \\ \frac{1}{2} \\ \\ \end{matrix}\right]$$

$$= \frac{2^{1+p-b}\sqrt{\pi}\ \Gamma(b)}{\Gamma(a)\Gamma\left(\frac{b-a}{2}\right)\Gamma\left(\frac{b-a+1}{2}\right)} \times$$

$$\times\Bigg[\Gamma(a-p)\sum_{r=0}^{p}\left\{(-1)^r\binom{p}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p}{2}\right)}\right\}+$$

$$+\frac{(3+3c+d)(1-a+p)\Gamma(a-p-1)}{cd}\times$$

$$\times\sum_{r=0}^{p+2}\left\{(-1)^r\binom{p+2}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p-2}{2}\right)}\right\}+$$

$$+\frac{(9+3c+3d)(1-a+p)(2-a+p)\Gamma(a-p-2)}{cd(d+1)}\times$$
$$\times\sum_{r=0}^{p+4}\left\{(-1)^r\binom{p+4}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p-4}{2}\right)}\right\}+$$

$$+\frac{(1-a+p)(2-a+p)(3-a+p)(9+c+3d)\Gamma(a-p-3)}{cd(d+1)(d+2)}\times$$
$$\times\sum_{r=0}^{p+6}\left\{(-1)^r\binom{p+6}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p-6}{2}\right)}\right\}+$$

$$+\frac{(1-a+p)(2-a+p)(3-a+p)(4-a+p)\Gamma(a-p-4)}{cd(d+1)(d+2)}\times$$
$$\times\sum_{r=0}^{p+8}\left\{(-1)^r\binom{p+8}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p-8}{2}\right)}\right\}\Bigg],$$
(3.7)

where $a,b,c,d,b-a,a-p\in\mathbb{C}\setminus\mathbb{Z}_0^-$ and $p\in\mathbb{Z}_0$.

The proof of theorems (3.2)-(3.4) would be accomplished by following the lines of that of theorem (3.1) with the aid of summation theorem (1.6). The involved details are omitted.

4- Summation formulas for ${}_5F_4\left[\frac{1}{2}\right]$ and ${}_6F_5\left[\frac{1}{2}\right]$

Theorem 4.1. *The following summation theorem holds true*:

$${}_5F_4\left[\begin{matrix}a,1-a+p,c+1,d+1,g+1;\\ b,c,d,g;\end{matrix}\ \frac{1}{2}\right]$$
$$=\frac{2^{1+p-b}\sqrt{\pi}\ \Gamma(b)}{\Gamma(a)\Gamma\left(\frac{b-a}{2}\right)\Gamma\left(\frac{b-a+1}{2}\right)}\times$$
$$\times\Bigg[\Gamma(a-p)\sum_{r=0}^{p}\left\{(-1)^r\binom{p}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p}{2}\right)}\right\}+$$
$$+\frac{(1+c+d+g+cd+cg+dg)}{cdg}\times$$
$$\times(1-a+p)\Gamma(a-p-1)\times$$
$$\times\sum_{r=0}^{p+2}\left\{(-1)^r\binom{p+2}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p-2}{2}\right)}\right\}+$$

$$+\frac{(3+c+d+g)(1-a+p)(2-a+p)\Gamma(a-p-2)}{cdg}\times$$
$$\times\sum_{r=0}^{p+4}\left\{(-1)^r\binom{p+4}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p-4}{2}\right)}\right\}+$$
$$+\frac{(1-a+p)(2-a+p)(3-a+p)\Gamma(a-p-3)}{cdg}\times$$
$$\times\sum_{r=0}^{p+6}\left\{(-1)^r\binom{p+6}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p-6}{2}\right)}\right\}\Bigg],$$
(4.1)

where $a,b,c,d,g,b-a,a-p\in\mathbb{C}\setminus\mathbb{Z}_0^-$ and $p\in\mathbb{Z}_0$.

Proof of Theorem 4.1.**:** In order to establish the result, we proceed as follows.

$${}_5F_4\left[\begin{matrix}a,1-a+p,c+1,d+1,g+1;\\ b,c,d,g;\end{matrix}\ \frac{1}{2}\right]$$
$$=\sum_{r=0}^{\infty}\frac{(a)_r(1-a+p)_r(c+1)_r(d+1)_r}{(b)_r(c)_r(d)_r}\times$$

$$\times \frac{(g+1)_r (1/2)^r}{(g)_r\, r!}$$

$$= \sum_{r=0}^{\infty} \frac{(a)_r (1-a+p)_r (1/2)^r}{(b)_r\, r!} \times$$

$$\times \left\{ 1 + \frac{(1+c+d+g+cd+cg+dg)r}{cdg} + \right.$$

$$+ \frac{(3+c+d+g)}{cdg} r(r-1) +$$

$$\left. + \frac{1}{cdg} r(r-1)(r-2) \right\}$$

$$= \sum_{r=0}^{\infty} \frac{(a)_r (1-a+p)_r (1/2)^r}{(b)_r\, r!} +$$

$$+ \frac{(1+c+d+g+cd+cg+dg)}{cdg} \times$$

$$\times \sum_{r=1}^{\infty} \frac{(a)_r (1-a+p)_r (1/2)^r}{(b)_r\, (r-1)!} +$$

$$+ \frac{(3+c+d+g)}{cdg} \sum_{r=2}^{\infty} \frac{(a)_r (1-a+p)_r (1/2)^r}{(b)_r\, (r-2)!} +$$

$$+ \frac{1}{cdg} \sum_{r=3}^{\infty} \frac{(a)_r (1-a+p)_r (1/2)^r}{(b)_r\, (r-3)!}. \quad (4.2)$$

Replacing r by $r+1$ in the second term, r by $r+2$ in the third term, and r by $r+3$ in the fourth term on the right hand side of the equation (4.2), we get

$${}_5F_4\left[\begin{matrix} a, 1-a+p, c+1, d+1, g+1; \\ b, c, d, g; \end{matrix} \; \frac{1}{2}\right]$$

$$= \sum_{r=0}^{\infty} \frac{(a)_{r+1} (1-a+p)_{r+1} (1/2)^{r+1}}{(b)_{r+1}\, r!} +$$

$$+ \frac{(1+c+d+g+cd+cg+dg)}{cdg} \times$$

$$\times \sum_{r=0}^{\infty} \frac{(a)_{r+1} (1-a+p)_{r+1} (1/2)^{r+1}}{(b)_{r+1}\, r!} +$$

$$+ \frac{(1+c+d+g+cd+cg+dg)}{cdg} \times$$

$$+ \frac{(3+c+d+g)}{cdg} \sum_{r=0}^{\infty} \frac{(a)_{r+2} (1-a+p)_{r+2} (1/2)^{r+1}}{(b)_{r+2}\, r!} +$$

$$+ \frac{1}{cdg} \sum_{r=0}^{\infty} \frac{(a)_{r+3} (1-a+p)_{r+3} (1/2)^{r+3}}{(b)_{r+3}\, r!}.$$

$$= \sum_{r=0}^{\infty} \frac{(a)_r (1-a+p)_r (1/2)^r}{(b)_r\, r!} +$$

$$+ \frac{(1+c+d+g+cd+cg+dg) a(1-a+p)}{2bcdg} \times$$

$$\times \sum_{r=0}^{\infty} \frac{(1+a)_r (2-a+p)_r (1/2)^r}{(1+b)_r\, r!} +$$

$$+ \frac{(3+c+d+g) a(1+a)(1-a+p)(2-a+p)}{4b(b+1)cdg} \times$$

$$\times \sum_{r=0}^{\infty} \frac{(2+a)_r (3-a+p)_r (1/2)^r}{(2+b)_r\, r!} +$$

$$+ \frac{a(1+a)(1-a+p)(2-a+p)(3-a+p)}{8b(b+1)(b+2)cdg} \times$$

$$\times \sum_{r=0}^{\infty} \frac{(3+a)_r (4-a+p)_r (1/2)^r}{(3+b)_r\, r!}$$

$$= {}_2F_1\left[\begin{matrix} a, 1-a+p; \\ b; \end{matrix} \; \frac{1}{2}\right] +$$

$$+ \frac{(1+c+d+g+cd+cg+dg) a(1-a+p)}{2bcdg} \times$$

$$= {}_2F_1\left[\begin{matrix} 1+a, 2-a+p; \\ 1+b; \end{matrix} \; \frac{1}{2}\right] +$$

$$+ \frac{(3+c+d+g) a(1+a)(1-a+p)(2-a+p)}{4b(b+1)cdg} \times$$

$$= {}_2F_1\left[\begin{matrix} 2+a, 3-a+p; \\ \\ 2+b; \end{matrix} \quad \frac{1}{2}\right] +$$

$$+\frac{a(1+a)(1-a+p)(2-a+p)(3-a+p)}{8b(b+1)(b+2)cdg}\times$$

$$= {}_2F_1\left[\begin{matrix} 3+a, 4-a+p; \\ \\ 3+b; \end{matrix} \quad \frac{1}{2}\right] \qquad (4.3)$$

Now applying the summation theorem (1.6) in equation (4.3), we get

$${}_5F_4\left[\begin{matrix} a, 1-a+p, c+1, d+1, g+1; \\ \\ b, c, d, g; \end{matrix} \quad \frac{1}{2}\right]$$

$$= \frac{2^{1+p-b}\sqrt{\pi}\ \Gamma(b)\Gamma(a-p)}{\Gamma(a)\Gamma\left(\frac{b-a}{2}\right)\Gamma\left(\frac{b-a+1}{2}\right)}\times$$

$$\times\sum_{r=0}^{p}\left\{(-1)^r\binom{p}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r}{2}\right)-p}\right\}+$$

$$+\frac{2^{1+p-b}(1-a+p)(1+c+d+g+cd+cg+dg)}{cdg\,\Gamma(a)}\times$$

$$\times\frac{\sqrt{\pi}\,\Gamma(b)\Gamma(a-p-1)}{\Gamma\left(\frac{b-a}{2}\right)\Gamma\left(\frac{b-a+1}{2}\right)}\times$$

$$\times\sum_{r=0}^{p+2}\left\{(-1)^r\binom{p+2}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\left(\frac{b+a+r+2}{2}\right)-(p+2)\right)}\right\}+$$

$$+\frac{2^{1+p-b}(1-a+p)(2-a+p)(3+c+d+g)}{cdg\,\Gamma(a)\Gamma\left(\frac{b-a}{2}\right)}\times$$

$$\times\frac{\sqrt{\pi}\ \Gamma(b)\Gamma(a-p-2)}{\Gamma\left(\frac{b-a+1}{2}\right)}\times$$

$$\times\sum_{r=0}^{p+4}\left\{(-1)^r\binom{p+4}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\left(\frac{b+a+r+4}{2}\right)-(p+4)\right)}\right\}+$$

$$+\frac{2^{1+p-b}(1-a+p)(2-a+p)}{cdg\,\Gamma(a)\Gamma\left(\frac{b-a}{2}\right)}\times$$

$$\times\frac{(3-a-p)\Gamma(a-p-3)\sqrt{\pi}\ \Gamma(b)}{\Gamma\left(\frac{b-a+1}{2}\right)}\times$$

$$\times\sum_{r=0}^{p+6}\left\{(-1)^r\binom{p+6}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\left(\frac{b+a+r+6}{2}\right)-(p+6)\right)}\right\}.$$

On simplifying further, we arrive at the result (4.1).

Theorem 4.2. *The following summation theorem holds true*:

:

$${}_5F_4\left[\begin{matrix} a, 1-a+p, c+1, d+1, g+2; \\ \\ b, c, d, g; \end{matrix} \quad \frac{1}{2}\right]$$

$$= \frac{2^{1+p-b}\sqrt{\pi}\ \Gamma(b)}{\Gamma(a)\Gamma\left(\frac{b-a}{2}\right)\Gamma\left(\frac{b-a+1}{2}\right)}\times$$

$$\times\left[\Gamma(a-p)\sum_{r=0}^{p}\left\{(-1)^r\binom{p}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p}{2}\right)}\right\}+\right.$$

$$\left.+\frac{(dg+cg+2cd+2c+2d+g+2)}{cdg}\times\right.$$

$$\times(1-a+p)\Gamma(a-p-1)\times$$

$$\times \sum_{r=0}^{p+2}\left\{(-1)^r\binom{p+2}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p-2}{2}\right)}\right\}+$$

$$+\frac{(1-a+p)(2-a+p)}{cdg(g+1)}\times$$

$$\times(10+cd+2dg+2cg+g^2+4c+4d+7g)\times$$

$$\times\Gamma(a-p-2)\sum_{r=0}^{p+4}\left\{(-1)^r\binom{p+4}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p-4}{2}\right)}\right\}+$$

$$+\frac{(1-a+p)(2-a+p)(3-a+p)}{cd}\times$$

$$\times\frac{(7+c+d+2g)\Gamma(a-p-3)}{g(g+1)}\times$$

$$\times\left\{\sum_{r=0}^{p+6}(-1)^r\binom{p+6}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p-6}{2}\right)}\right\}+$$

$$+\frac{(1-a+p)(2-a+p)(3-a+p)(4-a+p)\Gamma(a-p-4)}{cdg(g+1)}\times$$

$$\times\sum_{r=0}^{p+8}\left\{(-1)^r\binom{p+8}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p-8}{2}\right)}\right\}\Bigg],$$

(4.4)

where $a,b,c,d,g,b-a,a-p\in\mathbb{C}\setminus\mathbb{Z}_0^-$ and $p\in\mathbb{Z}_0$.

The proof of theorem (4.2) would run parallel to the theorem (4.1) with the help of summation theorem (1.6). The details are omitted.

Theorem 4.3. *The following summation theorem holds true*:

$${}_6F_5\left[\begin{matrix}a,1-a+p,c+1,d+1,g+1,h+1;\\ b,c,d,g,h;\end{matrix}\ \frac{1}{2}\right]$$

$$=\frac{2^{1+p-b}\sqrt{\pi}\ \Gamma(b)}{\Gamma(a)\Gamma\left(\frac{b-a}{2}\right)\Gamma\left(\frac{b-a+1}{2}\right)}\times$$

$$\times\left[\Gamma(a-p)\sum_{r=0}^{p}\left\{(-1)^r\binom{p}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p}{2}\right)}\right\}+\right.$$

$$+\frac{(1-a+p)\Gamma(a-p-1)(1+cdg+cdh+cgh+dgh+}{cd}$$

$$\left.\frac{+cd+cg+ch+dg+dh+gh+c+d+g+h)}{gh}\times\right.$$

$$\times\sum_{r=0}^{p+2}\left\{(-1)^r\binom{p+2}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p-2}{2}\right)}\right\}+$$

$$+\frac{(1-a+p)(2-a+p)(7+cd+cg+ch+dg+}{cd}$$

$$\frac{+dh+gh+3c+3d+3g+3h)\Gamma(a-p-2)}{gh}\times$$

$$\times\sum_{r=0}^{p+4}\left\{(-1)^r\binom{p+4}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p-4}{2}\right)}\right\}+$$

$$\times\frac{(6+c+d+g+h)(1-a+p)(2-a+p)(3-a+p)}{cdgh}\times$$

$$\times\Gamma(a-p-3)\sum_{r=0}^{p+6}\left\{(-1)^r\binom{p+6}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p-6}{2}\right)}\right\}+$$

$$+\frac{(1-a+p)(2-a+p)(3-a+p)(4-a+p)\Gamma(a-p-4)}{cdgh}\times$$

$$\left.\times\sum_{r=0}^{p+8}\left\{(-1)^r\binom{p+8}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\frac{b+a+r-2p-8}{2}\right)}\right\}\right],$$

(4.5) where

$a,b,c,d,g,h,b-a,a-p\in\mathbb{C}\setminus\mathbb{Z}_0^-$ and $p\in\mathbb{Z}_0$.

Proof of Theorem 4.3.**:** In order to establish the result, we proceed as follows.

$$
{}_6F_5\left[\begin{matrix} a,1-a+p,\ c+1,\ d+1,\ g+1,h+1; \\ \\ b,c,d,g,h; \end{matrix}\ \frac{1}{2}\right]
$$

$$
=\sum_{r=0}^{\infty}\frac{(a)_r(1-a+p)_r(c+1)_r(d+1)_r}{(b)_r(c)_r(d)_r}\times
$$

$$
\times\frac{(g+1)_r(h+1)_r(1/2)^r}{(g)_r(h)_r\,r!}
$$

$$
=\sum_{r=0}^{\infty}\frac{(a)_r(1-a+p)_r(1/2)^r}{(b)_r\,r!}\times
$$

$$
\times\left\{1+\frac{(1+cdg+cdh+cgh+dgh+cd+cg+}{cd}\right.
$$

$$
\frac{+ch+dg+dh+gh+c+d+g+h)}{gh}r+
$$

$$
+\frac{(7+cd+cg+ch+dg+dh+gh+}{cd}
$$

$$
\frac{+3c+3d+3g+3h)}{gh}r(r-1)+
$$

$$
+\frac{(6+c+d+g+h)}{cdgh}r(r-1)(r-2)+
$$

$$
\left.+\frac{1}{cdgh}r(r-1)(r-2)(r-3)\right\} \qquad (4.6)
$$

$$
=\sum_{r=0}^{\infty}\frac{(a)_r(1-a+p)_r(1/2)^r}{(b)_r\,r!}+
$$

$$
+\frac{(1+cdg+cdh+cgh+dgh+cd+cg+}{cd}
$$

$$
\frac{+ch+dg+dh+gh+c+d+g+h)}{gh}\times
$$

$$
\times\sum_{r=1}^{\infty}\frac{(a)_r(1-a+p)_r(1/2)^r}{(b)_r(r-1)!}+
$$

$$
+\frac{(7+cd+cg+ch+dg+d\,h+}{cd}
$$

$$
\frac{+gh+3c+3d+3g+3h)}{gh}\times
$$

$$
\times\sum_{r=2}^{\infty}\frac{(a)_r(1-a+p)_r(1/2)^r}{(b)_r(r-2)!}+
$$

$$
+\frac{(6+c+d+g+h)}{c\,d\,gh}\sum_{r=3}^{\infty}\frac{(a)_r(1-a+p)_r(1/2)^r}{(b)_r(r-3)!}+
$$

$$
+\frac{1}{c\,d\,g\,h}\sum_{r=4}^{\infty}\frac{(a)_r(1-a+p)_r(1/2)^r}{(b)_r(r-4)!} \qquad (4.7)
$$

Replacing r by $r+1$in the second term, r by $r+2$ in the third term, r by $r+3$ in the fourth term, and r by $r+4$ in the fifth term on the right hand side of the equation (4.7), we get

$$
{}_6F_5\left[\begin{matrix} a,1-a+p,\ c+1,\ d+1,\ g+1,h+1; \\ \\ b,c,d,g,h; \end{matrix}\ \frac{1}{2}\right]
$$

$$
=\sum_{r=0}^{\infty}\frac{(a)_r(1-a+p)_r(1/2)^r}{(b)_r\,r!}+
$$

$$
+\frac{(1+cdg+cdh+cgh+dgh+cd+cg+}{cd}
$$

$$
\frac{+ch+dg+dh+gh+c+d+g+h)\times}{gh}
$$

$$
\frac{\times a(1-a+p)}{2b}\sum_{r=0}^{\infty}\frac{(1+a)_r(2-a+p)_r(1/2)^r}{(1+b)_r\,r!}+
$$

$$
+\frac{(7+cd+cg+ch+dg+dh+gh+3c+3d+}{cdgh}
$$

$$
\frac{+3g+3h)a(a+1)(1-a+p)(2-a-p)}{4b(b+1)}\times
$$

$$
\times\sum_{r=0}^{\infty}\frac{(2+a)_r(3-a+p)_r(1/2)^r}{(2+b)_r\,r!}+
$$

$$
+\frac{a(a+1)(a+2)(1-a+p)(2-a+p)}{8b(b+1)(b+2)}\times
$$

$$
\times\frac{(3-a+p)(6+c+d+g+h)}{cdgh}\times
$$

$$
\times\sum_{r=0}^{\infty}\frac{(3+a)_r(4-a+p)_r(1/2)^r}{(3+b)_r\,r!}+
$$

$$
+\frac{a(a+1)(a+2)(a+3)(1-a+p)(2-a+p)}{16b(b+1)(b+2)(b+3)}\times
$$

$$
\times\frac{(3-a+p)(4-a+p)}{cd\ gh}\sum_{r=0}^{\infty}\frac{(4+a)_r(5-a+p)_r(1/2)^r}{(4+b)_r\,r!}
$$

$$= {}_2F_1\left[\begin{matrix} a, 1-a+p; \\ b; \end{matrix} \middle| \frac{1}{2}\right] +$$

$$+ \frac{(1+cdg+cdh+cgh+dgh+cd+cg+ch+}{2cd}$$

$$\frac{+cg+ch+dg+dh+gh+c+d+g+h)}{2bgh} \times$$

$$\times a(1-a+b)\,{}_2F_1\left[\begin{matrix} 1+a,\ 2-a+p; \\ 1+b; \end{matrix} \middle| \frac{1}{2}\right] +$$

$$+ \frac{(7+cd+cg+ch+dg+dh+gh+}{cd}$$

$$\frac{+3c+3d+3g+3h)a(a+1)(1-a+p)}{4b(b+1)gh} \times$$

$$\times \frac{a(a+1)(1-a+p)(2-a+p)}{4b(b+1)} \times$$

$$\times (2-a+p)\,{}_2F_1\left[\begin{matrix} 2+a,\ 3-a+p; \\ 2+b; \end{matrix} \middle| \frac{1}{2}\right] +$$

$$+ \frac{a(a+1)(a+2)(1-a+p)(2-a+p)}{8b(b+1)(b+2)} \times$$

$$\times \frac{(3-a+p)(6+c+d+g+h)}{cdgh} \times$$

$$\times {}_2F_1\left[\begin{matrix} 3+a,\ 4-a+p; \\ 3+b; \end{matrix} \middle| \frac{1}{2}\right] +$$

$$+ \frac{a(a+1)(a+2)(a+3)(1-a+p)(2-a+p)}{16b(b+1)(b+2)(b+3)} \times$$

$$\times \frac{(3-a+p)(4-a+p)}{cdgh}\,{}_2F_1\left[\begin{matrix} 4+a,\ 5-a+p; \\ 4+b; \end{matrix} \middle| \frac{1}{2}\right] \quad (4.8)$$

Applying the summation theorem (1.6) in equation (4.8), we get

$${}_6F_5\left[\begin{matrix} a, 1-a+p, c+1, d+1, g+1, h+1; \\ b, c, d, g, h; \end{matrix} \middle| \frac{1}{2}\right] =$$

$$= \frac{2^{1+p-b}\sqrt{\pi}\,\Gamma(b)\Gamma(a-p)}{\Gamma(a)\Gamma\left(\frac{b-a}{2}\right)\Gamma\left(\frac{b-a+1}{2}\right)} \times$$

$$\times \sum_{r=0}^{p} \left\{ (-1)^r \binom{p}{r} \frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\left(\frac{b+a+r}{2}\right)-p\right)} \right\} +$$

$$+ \frac{2^{1+p-b}(1-a+p)\sqrt{\pi}\,\Gamma(b)\Gamma(a-p-1)}{\Gamma(a)\Gamma\left(\frac{b-a}{2}\right)\Gamma\left(\frac{b-a+1}{2}\right)} \times$$

$$\times \frac{(1+cdg+cdh+cgh+dgh+cd+cg+}{cd}$$

$$\frac{+ch+dg+dh+gh+c+d+g+h)}{gh} \times$$

$$\times \sum_{r=0}^{p+2} \left\{ (-1)^r \binom{p+2}{r} \frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\left(\frac{b+a+r+2}{2}\right)-(p+2)\right)} \right\} +$$

$$+ \frac{2^{1+p-b}(1-a+p)(2-a+p)}{\Gamma(a)\Gamma\left(\frac{b-a}{2}\right)\Gamma\left(\frac{b-a+1}{2}\right)} \times$$

$$\times \frac{(7+cd+cg+ch+dg+dh+gh+3c+}{cd}$$

$$\frac{+3d+3g+3h)\sqrt{\pi}\,\Gamma(b)\Gamma(a-p-2)}{gh} \times$$

$$\times \sum_{r=0}^{p+4} \left\{ (-1)^r \binom{p+4}{r} \frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\left(\frac{b+a+r+4}{2}\right)-(p+4)\right)} \right\} +$$

$$+ \frac{2^{1+p-b}(1-a+p)(2-a+p)(3-a+p)}{cdgh\,\Gamma(a)\Gamma\left(\frac{b-a}{2}\right)} \times$$

$$\times\frac{(6+c+d+g+h)\,\Gamma(a-p-3)\Gamma(b)\sqrt{\pi}}{\Gamma\left(\frac{b-a+1}{2}\right)}\times$$

$$\times\sum_{r=0}^{p+6}\left\{(-1)^r\binom{p+6}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\left(\frac{b+a+r+6}{2}\right)-(p+6)\right)}\right\}+$$

$$+\frac{2^{1+p-b}(1-a+p)(2-a+p)(3-a+p)}{cdgh\,\Gamma(a)\Gamma\left(\frac{b-a}{2}\right)}\times$$

$$\times\frac{(4-a+p)\,\Gamma(a-p-4)\Gamma(b)\sqrt{\pi}}{\Gamma\left(\frac{b-a+1}{2}\right)}\times$$

$$\times\sum_{r=0}^{p+8}\left\{(-1)^r\binom{p+8}{r}\frac{\Gamma\left(\frac{b-a+r}{2}\right)}{\Gamma\left(\left(\frac{b+a+r+8}{2}\right)-(p+8)\right)}\right\}.$$

On simplifying further, we arrive at the result (4.5).

CONCLUSION

In our present investigation, we have given certain extensions and generalizations of Kümmer's third summation Theorem (1.5) in the form of ${}_3F_2[1/2]$, ${}_4F_3[1/2]$, ${}_5F_4[1/2]$ and ${}_6F_5[1/2]$ where some numerator and denominator parameters differ by a positive integer, as claimed in the above theorems. We conclude this paper with the remark that many other summation theorems can be derived in an analogous manner. Moreover, the results deduced above are expected to lead to some potential applications in several fields of applied mathematics, statistics, and engineering sciences.

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Production of High-Quality Tomato Seedlings in the Open Field Nurseries

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Seed rates;
Quality;
Dry weight;
Density.

Abstract: The aim of the study is to produce tomatoes seedlings with good characteristics for summer and fall seedlings seasons in field nurseries by investigating three plant distances (10, 15, 20 cm), inaddition to scatteringand two levels of seed rate (100 and 150% of the recommended rates).The experiment was carried out using completely random plots using split plot designs in three replications, and the levels of each factor were randomly distributed within the plots withcultivation of two seedbeds. Planting tomato seedlings at a 20 cm distance can improve tomato seedlings' growth in open field nurseries. Finally, more researches are needed to determine the optimal seed rates as well as planting distances in open field nurseries production under Al-Jabal Al-Khader conditions.

إنتاج شتلات الطماطم عالية الجودة في مشاتل الحقول المفتوحة

الكلمات المفتاحية :
مسافات الزراعة؛
معدلات البذور؛
الجودة؛
الوزن الجاف؛
الكثافة.

المستخلص : تستهدف هذه الدراسة إنتاج شتلات طماطم ذات خصائص جيدة لموسمي الصيف والخريف في المشاتل الحقلية، عن طريق اختبار ثلاث مسافات نباتية (10، 15، 20 سم) فضلًا عن الزراعة بالنثر ومستويين من البذور بمعدل 100 و 150 ٪ من المعدلات الموصى بها في المشتل الميداني المفتوح. نُفِّذت التجربة باستخدام القطاعات العشوائية الكاملة، باستخدام تصميم القطع المنشقَّة بثلاث مكررات. وتشير نتائج هذه الدراسة إلى أن زراعة شتلات الطماطم على مسافة 20 سم يمكن أن يحسِّن نمو شتلات الطماطم في مشاتل الحقول المفتوحة في المنطقة، علاوة على ذلك تشير النتائج إلى أن معدلات البذور في مشاتل الحقول المفتوحة ليست حاسمة لإنتاج شتلات الطماطم في المنطقة. أخيرًا، هناك حاجة إلى مزيد من البحوث لتحديد معدلات البذور المُثلى ومسافات الزراعة المناسبة في إنتاج المشاتل المفتوحة تحت ظروف منطقة الجبل الأخضر.

INTRODUCTION

Tomatoes (*Solanumlycopersicum*) have grown to be one of the world's most popular and extensively cultivated vegetable. In terms of total yearly world output, tomato ranks as one of the essential vegetables recognized by the Food and Agriculture Organization (FAO). Tomatoes are a very important vegetable crop in terms of economics, and they are farmed in 175 nations (FAO, 2014).

Between 2000 and 2019, the global production of vegetables increased more quickly, rising by 65 percent. Tomatoes are the most abundant of the five major vegetable species, making up between 42 and 45 percent of the total over the time period, and tomatoes represented about 16 percent in 2019 (FAO, 2021). China is the world's greatest tomato grower, with 52 million tons, then India, and United States is the third-largest producer,

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then Egypt, with 18, 14, and 8 million tons, respectively (FAO, 2014). Government revenue, smallholder farmers, and foreign exchange gains have all benefited from the horticultural sector of agriculture. Additionally, the sector contributes significantly to food security and is a critical supplier of raw materials for the industrial sector (Irungu, 2011).

Development of profitable tomatoes is a huge difficulty challenge all over the world. Tomatoes plants can be grown in the field directly through plug-mix sowing (Hayslip, 1974), or by transplanting (Hochmuth, 1988). Tomato seedlings have a better chance of surviving, establishing quicker, improving plant uniformity, and maturing sooner than immediately seeded plants (Weston & Zandstra, 1989). Depending on the available planting space, operational simplicity, and adaptability of the approach, seedlings may be raised in trays, beds, or seedboxes (Lin et al., 2015). However, even in new and modern trays, seedlings could root and branch into the tray, which leads to poor removal and extraction of the plug, in addition, it is difficult to keep the seed boxes or trays clean (Balliu et al., 2017). Brower (1963) explained a functional balance between roots and shoots as connected growth, in which changes in shoot growth rate are reflected in the roots and inversely, therefore, to prevent these difficulties, it is preferable to grow tomato seedlings on beds in a field nursery rather than utilizing containers like seed-boxes or trays to produce seedlings. Properly grown transplants have an impact on vegetable output. Successful vegetable cultivation depends heavily on healthy seedlings generated in an expert nursery, especially for transplanted crops like tomatoes (Lin et al., 2015). Transplants are considered good quality when they are free of disease and pest infections, have the potential to live in difficult settings after transplanting, have a good root system, and contain a leaf area without any visible problems (Weston & Zandstra, 1989). Air humidity, CO2, culture techniques, light, Temperature, water availability, all of which are directly impacted by the density of seedlings, which is represented in the number of seeds, including the planting space between rows, where fragile and weak seedlings may emerge as a result of shading and then have an impact on the quality of transplants (Brazaitytė et al., 2010; Juknys et al., 2011; Lin et al., 2015; Paul & Metzger, 2005).A vegetable nursery is a location or facility where immature vegetable seedlings are raised or handled until they are suitable for more permanent planting (Bharathi & Ravishankar, 2018; Hassan, 1991), demonstrates that field nurseries must be in a pest-free environment so that pests do not attack seedlings, which are then transported to the permanent field. To avoid any harm, insecticides must be applied to the nursery site. According to the degree of levelness of the terrain in the field, field nurseries are constructed in the shape of beds with an area of 1 x 1, 2 x 2, or 2 x 3 meters. Planting in lines is ideal, as long as the spacing between them is 15-20 cm and the seeds are planted at a depth of 1.5 to 2 cm. Although heavy soils are not advised for nurseries, they can be utilized if required by covering the seeds with a mixture of sand and gravel. In the other hand, to tackle the management issues that nursery farmers encounter, further study is needed.

Nurseries had received no training, and various technical issues were discovered, affecting their profitability as well as the quality of seedlings delivered to farmers. Because the majority of crop varieties were open-pollinated, farmers used to generate their own seedlings for transplanting at a reduced cost. To boost output, most commercial farmers are turning to intensive vegetable growing with high-yielding F1 hybrids. Because these hybrid seeds are so expensive, it's critical to turn each seed into a healthy seedling, which necessitates meticulous nursery care. In most progressing nations, vegetable seedling production is done by specialist farmers or companies (Bharathi & Ravishankar, 2018).

Seeding rate and plant density, based to (Yucel, 2013), are major factors impacting

vegetable crop output and quality. Increased sowing rates, according to the same scientist, may improve agricultural competitiveness. Crop profitability, on the other hand, may or may not improve as a result of the high seed cost. However, there were no significant impacts of plant densities on pea plant height and other examined attributes, according to (Barary et al., 1996; Ibrahim et al., 2019). In Agadir, Morocco, a study was conducted where three separate plant densities of two processing tomato varieties (Heinz 1370 and Rio Grande) were examined over two seasons in sandy loam soil (Elattir, 2002). The spacing separated between single seed lines and between seed line clusters were 1.3 meters and 0.25 meters, respectively. Seedlings were reduced to one, two, or three plants per clump at the second true-leaf phase, resulting in plant densities of 30400, 60800, and 91200 plants.ha^{-1}, correspondingly. With increasing plant density, the number of clusters per m^2 grew dramatically, with no differences across varieties. The maximum plant density enhanced yield by 40% as compared to the control (30400 plants. ha-1), with no significant variation across cultivars. Under high plant density, the Rio Grande variety produced a significant early yield. When the plant density rose, the average fruit weight fell. Researches are currently intended to familiarize tomato producers with effective nursery management procedures for developing healthy tomato seedlings and to encourage the use of healthy seedling preparation strategies to improve tomato yields. So, the aim of this study is to produce tomatoes seedlings with good characteristics for summer and fall seedlings seasons in field nurseries by investigating three plant distances and two levels of seed rate in the field nursery at Omar Al-Mukhtar University, Al-Jabal Al-Khader, Libya.

MATERIALS AND METHODS

The study was conducted at the farm of the Department of Horticulture, Faculty of Agriculture, Omar Al-Mukhtar University, Al-Jabal Al-Khaderin 2016 to investigate the impact of seeding rates and planting distances and the interaction between them on germination, vegetative growth and quality of tomato cv. Rio Grande **(**Syngenta , Cairo, Egypt**)** seedlings. Two seed rates of 100 and 150% of the recommended rates were used as well as three planting distances (10, 15, 20 cm) between the lines in addition to the scattering treatment. The nursery site soil characteristics display in Table 1.

Table: (1). Soil characteristics and properties.

Measurements		
Particle Size distribution	Sand (%)	14.25
	Silt (%)	51.15
	Clay (%)	34.6
Organic Matter (%)		2.3
E.C (Mmhos/ cm)		1.36
Total Nitrogen (%)		0.21
Soil pH		7.87
CO_3^{--}%		1.35
P ppm		115

Data collected at the region of the Department of Horticulture, Faculty of Agriculture, Omar Al-Mukhtar University

Experimental Design: The experiment was carried out using completely random plots using split plot designs in three replications, where the main plots were assigned to the two seed rates (100 and 150% of the recommended rates), and the sub-plots to the coefficients of planting distances (10, 15, 20 cm) and addition to scattering. The levels of each factor were randomly distributed within the plots with cultivation of two seedbeds, so that each replicate included 8 factorial treatments (2 seeding rate * 4 planting distances).

Fieldwork: 3 beds with dimensions of 4x 1m are well-equipped, and the weight of the seeds needed for planting in 1 m was prepared and calculated, as well as the weight of the seeds needed for planting per square meter. Beds were planned according to the studied planting distances and were distributed randomly within the experimental units. Three lines were planted in each experimental unit, and after irrigation, the beds were covered with plastic strips until germi-

nation was complete. The service operations were carried out by irrigation and purification of weeds. The seedlings were also fertilized by spraying with urea solution (150 mg N/L).After 45 days of planting, samples were taken (5 seedlings always from the middle line).

Studied Traits: The response of the seedlings to the effect of the treatments under study was estimated on 5 seedlings that were randomly selected from the middle line of each treatment in the three replicates, then the following measurements were recorded as an average of five representatives: Fresh and dry weight for seedlings, leaves and stems, number of days for germination, number of leaves/ plant, seedlings height, and chlorophyll content. Total chlorophyll content of leaves (100 mg) was determined by extracting chlorophyll by acetone (80%) and quantified by absorption spectrometry using a tomographic analyzer at wavelengths (645, 653, 666) nm (Laval-Martin, 1979).

Data Analysis: JMP was used to examine all of the data (Version 11.0 for Windows; SAS Institute, Cary, NC). In all cases, Levene's test was used to determine homogeneity of variances, and the Shapiro-Wilk test was used to confirm normality (W> 0.80). By employing square root or log transformations, certain data were changed before analysis to fulfill the requirements of normality and equality of variance. The LSMeans Student's test was used to compare treatment means at 0.05.

RESULTS AND DISCUSSION

Fresh and dry weight of seedlings, leaves, and stems were not significantly affected by seed rates (100 and 150% of the recommended rates) investigated in this experiment (Table 2).The dry matter weight of seedlings was significantly higher for the scattering treatment than the other planting distances investigated in the current study. This outcome might have been caused by the enhanced seedling growth at the other broadening planting distances, which had relatively easy access to environmental resources such as water, light and essential minerals than those in a scattering population. This, in turn, probably led to higher water content and a lower accumulation of dry matter and may have been the cause of the observed results. From another angle, this finding can be supported by the hypothesis of dilution (Greenwood et al., 1990), where increasing planting spacing, resulted in higher water content and lower dry matter accumulation.

Number of days for germination was affected by seed rates as well as planting distances examined in the current study, where the treatment of scattering differed from the rest of the other treatments, as it achieved the least number of days for germination, as well as with 150% of the recommended rates (Figure 1). where the crowding of the seeds with each other in the scattering treatment enhances their cooperation with each other in raising the soil cover and increasing the speed of their germination more than the seeds that are less crowded in the other studied treatments, likewise, number of seeds can show considerable variation within germination, since increasing the number of seeds is often associated with the highest survival. This finding concurred with that of (Elattir, 2002) in which under high plant density, the Rio Grande variety produced a significant early yield, in other words, the maximum plant density enhanced yield by 40% as compared to the control, and thus, this finding may be an important consideration for developing a management plan and addressing the early tomato production issues at Al-Jabal Al-Khader area.

Table(:2). Mean fresh and dry weight for the tomato seedlings cv. Rio Grande, leaves and stems, under different seed rates and different planting distances were used as well as the scattering treatment.

Seedling Weight[z]						
Weight (g)	Seed rate[y]	scattering	10 cm	15 cm	20 cm	average
Fresh	First rate	1.30 a[x]	1.32 a	1.02 a	0.96 b	1.04 A
	Second rate	1.00 a	1.04 a	1.10 a	1.09 a	1.06 A
	Average	1.15 A	1.18 A	1.06 A	1.03 A	NA
Dry	First rate	0.30 a	0.25 a	0.29 ab	0.19 b	0.25 A
	Second rate	0.35 a	0.29 ab	0.25 ab	0.31 a	0.30 A
	Average	0.33 A	0.27 B	0.27 B	0.25 AB	NA
LEAVES						
	Seed rate	scattering	10 cm	15 cm	20 cm	average
Fresh	First rate	0.74 a	0.77 a	0.68 a	0.91 a	0.77 A
	Second rate	0.92 a	0.54 b	0.86 a	0.54 b	0.72 A
	Average	0.83 A	0.65 B	0.76 A	0.72 A	NA
Dry	First rate	0.24 a	0.17 ab	0.25 a	0.26 a	0.23 A
	Second rate	0.25 a	0.26 a	0.14 b	0.23 a	0.22 A
	Average	7.10 A	0.23 A	0.20 A	0.25 A	NA
STEM						
	Seed rate	scattering	10 cm	15 cm	20 cm	average
Fresh	First rate	0.47 a	0.46 b	0.47 a	0.32 ab	0.33 A
	Second rate	0.32 ab	0.34 ab	0.18 b	0.19 ab	0.25A
	Average	0.40 A	0.20 B	0.33 A	0.26 AB	NA
Dry	First rate	0.20 a	0.14 b	0.10 b	0.11 b	0.14 A
	Second rate	0.10 b	0.10 b	0.12 b	0.14 b	0.12 A
	Average	0.16 A	0.12 A	0.11 A	0.12 A	NA

[z]All data were subjected to analysis of variance using ANOVA in JMP (version 11.0 for Windows; SAS Institute, Cary, NC).
[y]Seed rates are100% (First rate) and 150% (Second rate) of the recommended rates were used as well as three planting distances (10, 15, 20 cm) between the lines in addition to the scattering treatment (control).
[x]Means followed by the same letter within sampling date are not significantly different at $P<0.05$.

The main factor seed rates evaluated in this experiment had no significant effect on the total number of leaves per seedling (Figure2). However, it tended to be significantly different due to the planting distances tested in the current experiment, wherewith the 20 cm planting distance the total number of leaves per seedling was greater than the other distances investigated in this study. The greater number of leaves per seedling with 20 cm planting distance evaluated in the current experiment is likely owing to the plants in a wider spacing having easier access to environmental resources included water, light, and nutrients than those in a denser population (Berhane et al., 2016). There was no significant difference between seed rates as well

as planting distances tested in this research for seedlings height (cm) (Figure 3).

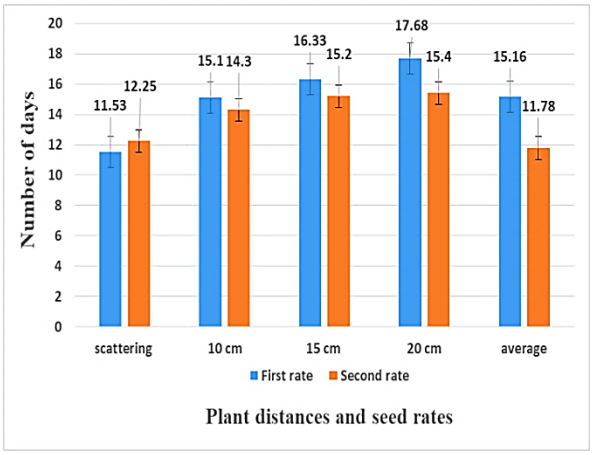

Figure: (1). Number of days for germination tomato seedlings cv. Rio Grande under different seed rates and different planting distances were used as well as the scattering treatment.

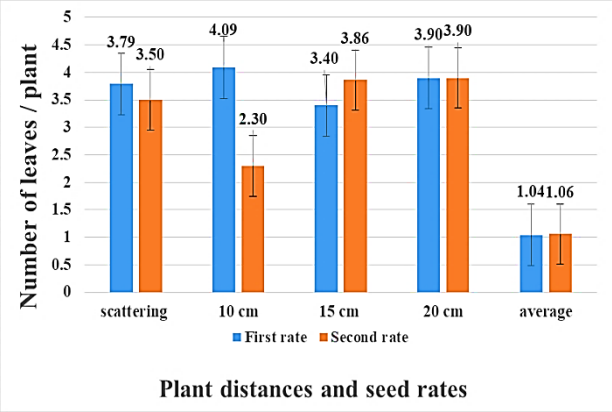

Figure: (2). Number of leaves per plant for tomato seedlings cv. Rio Grandeunder different seed rates and different planting distances were used as well as the scattering treatment.

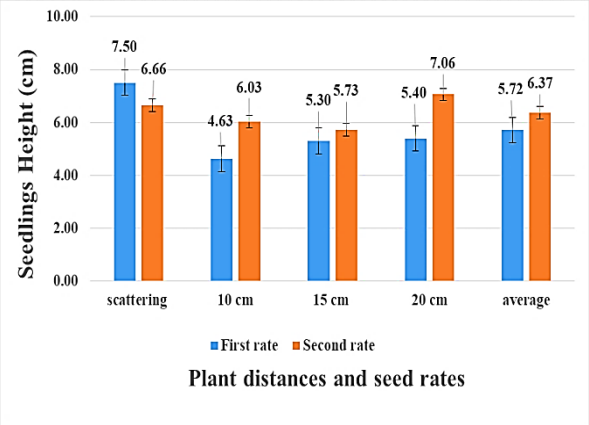

Figure: (3). Plant height for tomato seedlings cv. Rio Grande under different seed rates and different planting distances were used as well as the scattering treatment.

This finding is similar to (Barary et al., 1996; Ibrahim et al., 2019) that found there were no significant impacts of plant densities on pea plant height and other examined attributes. Chlorophyll content for tomato seedlings examined in this experiment was not significantly affected by seed rate treatment, but it was higher for 20 cm planting distance as compared to the other planting distances investigated in the current study (Figure 4). As previously explained is likely owing to the plants in a wider spacing having easier access to environmental and natural resources such as water, light, and nutrients than those with more population density (Berhane et al., 2016), suggesting that chlorophyll content analysis is a sensitive indicator of plant nutrient status, and maybe a useful and efficient tool to evaluate tomato seedlings nutrients status.

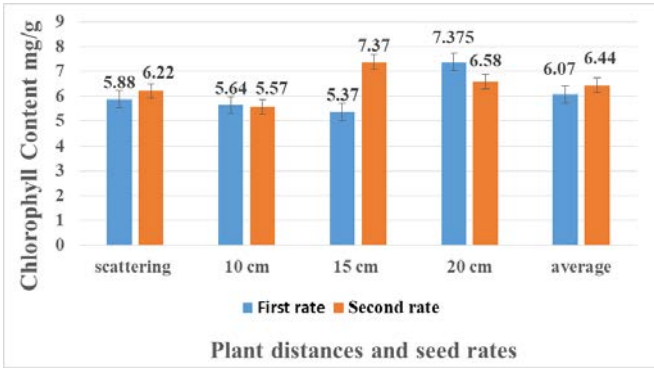

Figure: (4). Chlorophyll content for tomato seedlings cv. Rio Grandeunder different seed rates and different planting distances were used as well as the scattering treatment.

CONCLUSION

This result suggests that planting tomato seedlings at a 20 cm distance can improve tomato seedlings' growth in open field nurseries in the region by enhancing access to natural and environmental supplies including water, sunshine, and nutrition. This finding may be an important consideration for developing an annual management plan and addressing long-term issues for tomato seedlings production in open nurseries in Al-Jabal Al-Khader area. Further, results from the present study indicate that the seed rates in an open field nursery are not critical for tomato seedlings production in the region. As tomato is commonly grown in the whole world, and demand for tomato seedlings is increasing each year in Libya, more research is needed to determine the optimal seed rates as well as planting distances in open field nurseries production under Al-Jabal Al-Khader conditions.

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Author contributions: Fatma Mohamed conceived and designed the study, and supervised the fieldwork with support and help from Fayrouz Buojaylah and Alsunousi Masoud. Fayrouz Buojaylah took the lead in writing the manuscript in consultation with Fatma Mohamed and Alsunousi Masoud. Alsunousi Masoud performed the measurement and collected the data with support from Fayrouz Buojaylah and Fatma Mohamed. All the authors involved in this manuscript performed the laboratory work, data analysis, and approved the final version of the manuscript together.

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Bacterial Evaluation of Fresh Juices Sold in Cafes and Restaurants in the City of Benghazi, Libya

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Abstract: Food safety is a big challenge that concern all human communities and food production factories. The information on the bacterial evaluations of fresh natural juices in the city of Benghazi remained very limited and unclear. This study was carried out to evaluate the bacterial contamination for two of the most popular selling freshly made juices, including mango and strawberry juices. The results of screening showed bacterial growth in 117 (91.41%) out of the total samples 128. The evaluation during the second season showed a higher bacterial growth rate of 96.55 %. The most predominant bacteria contaminating the targeted juices was *Klebsiella pneumoniae*, with an average of (36.21%), followed by fecal *Escherichia coli* (31.03%) for both juices. All the contaminated juices represented bacteria count out of the accepted value. Gram-negative pathogens showed resistance to limited antibiotics, including Amoxicillin, Amikacin, and Clarithromycin, whereas the Gram-positive bacteria showed higher resistance rates. This study concluded that, increase in bacterial diversity and bacterial count out of the accepted standard as well as the presence of fecal *E. coli* indicating a poor level of hygiene during the process of preparing juices, leading to the possibility of causing diseases and exposing people to food poisoning.

التقييم البكتيري للعصائر الطبيعية الطازجة المباعة في مقاهي ومطاعم مدينة بنغازي، ليبيا

الكلمات المفتاحية:
الامن الغذائي
تلـ .وث العصـ .ائر الطبيعية
بكتيريـ .ا *E. coli* البرازية
التعداد الميكروبي

المستخلص: تعتبر سلامة الغذاء من التحديات الكبيرة التي تشغل بال كل المجتمعات البشرية ومصانع إنتاج الغذاء بشكل مستمر. ظلت المعلومات الخاصة بالتقييمات البكتيرية للعصائر الطبيعية الطازجة في مدينة بنغازي محدودة للغاية وغير واضحة. أجريت هذه الدراسة لتقييم التلوث البكتيري لاثنين من أكثر العصائر الطازجة متضمنة كلا من عصائر المانجو والفراولة. أظهرت نتائج الفحص نمو بكتيري في 117 (91.41٪) من مجموع العينات 128. أظهر التقييم خلال الموسم الثاني معدل نمو بكتيري أعلى بلغ 96.55٪. كانت البكتيريا الأكثر انتشارا هي بكتيريا *Klebsiella pneumoniae* بمتوسط (36.21٪)، تليها بكتريا *Escherichia coli* البرازية (31.03٪) لكلا نوعي العصائر. أظهرت النتائج أن جميع العصائر الملوثة بالبكتيريا هي خارج قيمة التعداد الميكروبي المقبول. أظهرت نتائج اختبار حساسية المضادات الحيوية أن البكتيريا سالبة الجرام مقاومة للمضادات الحيوية المحدودة، تتضمن كلا من Amoxicillin، Amikacin، Clarithromycin، بينما أظهرت البكتيريا موجبة الجرام معدلات مقاومة أعلى. وخلصت هذه الدراسة إلى أن زيادة التنوع البكتيري والعدد البكتيري هو خارج عن المعيار المقبول وكذلك وجود بكتيريا *E. coli* البرازية مما يشير إلى ضعف مستوى النظافة أثناء عملية تحضير العصائر مما يؤدي إلى إمكانية التسبب في الأمراض وتعريض الناس للتسمم الغذائي.

INTRODUCTION

Food safety is an important issue alarmingly concerned by all human communities and food production factories. Lack of the food hygiene and food sanitary quality direct of these products to be contaminated with several pathogenic bacteria leading to risk of foodborne illness

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(Gizaw, 2019; Ncama et al., 2021). Natural juices are among the food products that people drink a lot specially during the summer, as they are refreshing and rich in nutrients, however they are exposed to many pollutants, especially microbial contamination during the preparation process. Many of people working in the field of juices preparation do not have enough knowledge on the food safety and some do not follow the safety roles to avoid potentially severe health hazards, therefore the possibility of microbial contamination is very likely to occur (Ho et al., 2020; Ruxton et al., 2021). Several pathogenic bacteria are more frequent that combined with the juice contamination including *Escherichia coli*, *Staphylococcus aureus*, *Cryptosporidium*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Bacillus cereus*, *Salmonella sp.* and *Acinetobacter sp*. (Callejón et al., 2015; Hossen et al., 2020; Kader et al., 2014; Reddy et al., 2009; Tambekar et al., 2009).

The information on the microbial evaluations of fresh natural juices in the City of Benghazi are remained very limited and unclear. This project was carried out to evaluate the bacterial contamination into two of the most popular selling of freshly made juices including mango juice and strawberry juice in order to encourage people to spread awareness on food safety management and ways to combat foodborne illness.

MATERIALS AND METHODS

Study area and samples collection: The study was performed to evaluate the bacterial contamination of natural juices sold in the city of Benghazi. The samples collection targeted the most popular sale points including local fresh juice shops, cafes and restaurants and for the most popular sale products including mango and strawberry juices. In order to make sure the study cover the whole city areas, the collection of samples was designed to be including North, South, East, and the City Center within two seasons of collection. The investigation was carried out for 128 of fresh fruit juice samples served into plastic containers collected within two seasons as 64 sample of each. Within each season the microbial isolations were applied for 32 strawberry juice and 32 mango juice samples. After each collection, the samples were preserved into cold conditions and immediately transported to the laboratory for microbiological investigation.

Samples processing and culture conditions: In order to perform initial isolation and screening for bacterial contamination, all the fruit samples were first homogenized and under aseptic conditions one milliliter from each sample was introduced on Nutrient agar plate and MacConkey agar the plates were then incubated aerobically at 37 °C for overnight. Next day, all the plated were checked for bacterial growth and determine the diversity of bacterial types that involved the contamination. The bacterial cultures that showed more than one type of bacteria were processed for sub-culture step in order to obtain pure microbial cultures. All the well-isolated bacteria were preserved refrigerated at 4 °C using a slant Nutrient agar tubes prior bacterial identification and their susceptibility to the antibiotics.

Determination of the bacterial load and the most probable number that contaminate the juice samples: Serial dilutions using a sterile normal saline were made from each type of juice to make appropriate juice dilution concentrations of (10^{-1} up to 10^{-3}), one milliliter from each dilution was introduced and spread on Nutrient agar plate, the plates were then incubated aerobically at 37 °C for overnight. Next day, all the plated were checked to determine the bacterial colony forming unite (cfu/ml) according to the following formula: Number of bacteria colonies in the original sample = Number of colonies counted x 1/df x V (ml). Where: df, Dilution factor; V, Volume in ml and compared with the standard bacterial count (Asghar et al., 2018). For the MPN method, the analysis was performed in three steps including presumptive test, confirmatory test and completed test.

Identification of the isolated bacteria: After bacterial purification, the bacterial cultures were directed for morphological characterization including colonies differences on the agar plates such as shape, size and color of the colonies, lactose fermentation, and blood hemolysis, followed by microscopic morphology examination to investigate the differences between bacterial cells by using Gram staining techniques. Further assessments were used for bacterial identification including several traditional biochemical tests by using lactose fermentation, glucose fermentation, sucrose fermentation, gas production and using different culture media including MacConkey agar, Blood agar, Eosin methylene blue agar (EMB), Lactose Broth, Selenite Broth, Salmonella Shigella agar (SS), Triple sugar iron agar (TSI), Simmon`s citrate agar, Urease agar, DNase agar and Mannitol salt agar (MSA).

Some enzymatic chemical test also used to determine and differences between microbes including oxidase test, catalase test, and coagulate test. In order to investigate presence of fecal coliform, the samples that showed presence of *E. coli* growth were checked for positivity to the gas production and then incubated at 44.5 °C for 24–48 hrs. For conformation of the bacterial identification, Automated Phoenix 100 System for bacterial identification was used.

Antibiotic susceptibility testing of the bacteria contaminating fresh juices: All the identified bacteria tested for their sensitivity to the antibiotics in order to determine the antibiogram profile that contributing the multidrug resistant pathogens. This test was performed using the Kirby–Bauer disc diffusion method using Muller Hinton agar (MHA) plates as described by Clinical and Laboratory Standards Institute (CLSI, 2006) as follow: a bacterial suspension was prepared according to the 0.5 McFarland standard using a sterile normal saline and the turbidity was adjusted such that it contained approximately 1×10^6 cfu/ml.

The prepared bacterial suspension was smeared onto the Muller Hinton agar (MHA) plates using a sterile cotton swab followed by selection of known concentration of antibiotic-disks purchased from (Oxoid) including Amikacin (5 µg), Amoxicillin (10 µg), Cefixime (5 µg), Cefuroxime (30 µg), Cephalexin (30 µg), Ciprofloxacin (5 µg), Clarithromycin (15 µg), Doxycycline (30 µg), Imipenem (10 µg), Oxacillin (1 µg) and Sulfamethoxazole-Trimethoprim (25 µg) were placed onto the plates, after that the plates were incubated aerobically at 37 °C for overnight. Next day, the effect of the antibiotics was evaluated as a clear inhibition zone formed arrowed the antibiotic discs measured in millimeter of diameter.

RESULTS

The assessment was carried out according to increase the sources and the risk factors that represent a possible source of contamination with bacterial pathogens leading to health problems. The bacterial screening results for strawberry and mango juices showed that 117 (91.41%) of the total samples were contaminated with bacteria, the Gram-negative bacteria were representing the most predominant 110 (94%) out of 117 selected isolates.

The distribution of isolated bacteria according to the season and time of isolation was showing microbial growth at first isolation 58 (90.62%) out of 64 samples and 56 (96.55%) out of the 58 samples, were showing Gram-negative bacteria. During the second season, the results of bacterial screening showed 59 (92.19%) out of 64 samples were contaminated with bacteria, as well as 54(91.53%) of the isolated pathogens were classified belong to Gram-negative bacteria, figure (1).

Frequency of bacterial contamination according to the area: In order to cover the whole study area, the samples were collected from the four geographic areas of the City of Benghazi, including North, South, East and city Center. Within each geographic area, four targeted collection areas were included. From

each collection area, four collection points were targeted. From each collection point, two samples were collected during the two seasons.

The results showed high bacterial growth into the four geographic areas. For the North area, the bacterial growth was into 93.75% and 100% of the samples during the first and second seasons respectively. For the South area, the bacterial growth was into 93.75% and 81.25% of the samples during the first and second seasons respectively. For the East area, the bacterial growth was into 100% and 87.50% of the samples during the first and second seasons respectively. For the City Center area, the bacterial growth was into 87.50% of the samples for both the first and the second seasons, table (1).

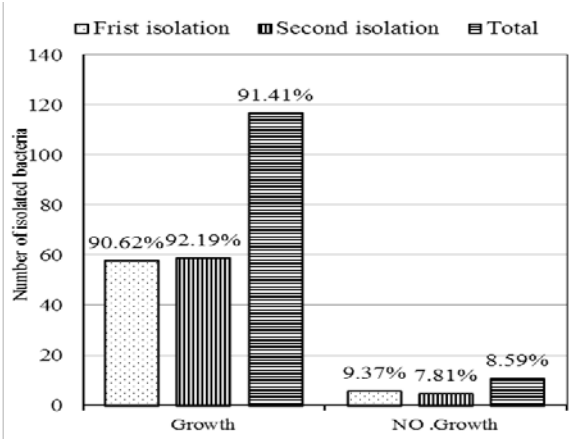

Figure (1): Shows the general bacterial investigation during the two seasons of collection representing total bacterial growth**.**

Diversity of the bacterial pathogens associated with the four studied areas: In order to determine the most predominant microbial contaminant in each geographic studied area, the isolated bacteria were assessed for identification, the results showed that *Klebsiella pneumoniae* represented the most predominant pathogen among all species, where it isolated from 42 (35.89%) juice sample, followed by fecal *E. coli* 36 (30.76 %), *Pseudomonas aeruginosa* 15 (12.82 %), *E. coli* 9 (7.69 %), *Enterobacter aerogenes* 8 (6.84 %), *Staphylococcus aureus* 5 (4.27 %) and *Staphylococcus schleiferi* 2 (1.71%).

The distribution of the isolated bacteria according to the geographical area separately showed that, in the North area, the most frequent isolated contaminant was fecal *E. coli* 12 (37.50%) followed by *K. pneumoniae* 8 (25%). In the South area, *P. aeruginosa* 10 (31.25 %) represented the most frequent bacteria followed by *K. pneumoniae* 9 (28.13%). In the East area, the most frequent isolated contaminant was fecal *E. coli* 12 (37.50%) followed by *K. pneumoniae* 9 (28.13%). In the City Center area, the most frequent isolated contaminant was *K. pneumoniae* 16 (50%) followed by fecal *E. coli* 6 (18.75%), table (2).

Distribution of bacterial species according to the types of juices: The results showed the contamination with *K. pneumoniae* represented the most predominant microbe contaminating the mango juice 23 (54.76%) and 19 (45.24%) for strawberry juice. The juice contamination with fecal *E. coli* showed 22 (61.11%) isolates into the mango juice and 14 (38.89%) isolates for the strawberry juice. The juice contamination with *P. aeruginosa* showed 8 (53.33%) for the strawberry juice and 7 (46.67%) mango juice. The results also showed the both juices have contaminated with some other different microorganisms at low rate of bacterial percentage as shown in the figure (2).

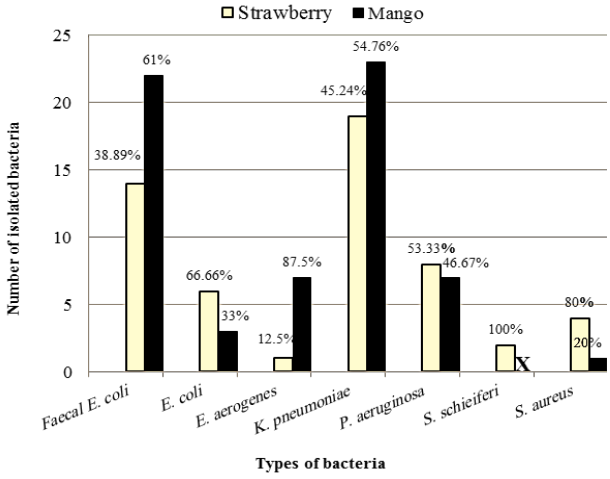

Figure (2): Distribution of the bacterial species according to the types of juices

Direct measurement of microbial growth through determination of most probable number: In order to investigate the most probable number for the bacteria that contributing the bacterial infection risk factor, a direct measurement of bacterial count on plate agar was performed through serial dilutions were made from the two type of juices; the results of growth were compared with the standard bacterial count chart provided by the recommended Gulf standard[11] that instructed the mean total viable count (microbial load) should be in all the freshly prepared fruit juices in the range of $0.1x10^2$ to $1.48x10^3$ cfu/ml, where the results showed that, *P. aeruginosa* revealed the highest rate of bacterial average count of $1.484x10^3$ cfu/ml, followed by fecal *E. coli* that showed average of count of $1.228x10^3$ cfu/ml, *K. pneumoniae* with an average of count $9.70x10^2$ cfu/ml, *E. aerogenes* showed $5.74x10^2$ cfu/ml, *E. coli* showed $2.7x10^2$ cfu/ml, and the contamination with *S. aureus* and *S. schleiferi* showed low bacterial count of $0.7x10^2$ cfu/ml and $0.1x10^2$ cfu/ml, figure (3).

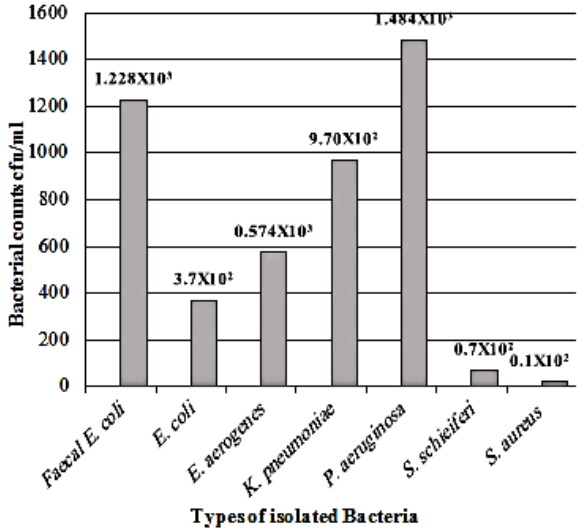

Figure (3): Bacterial counts (cfu/ml) into the both fresh juices

Antibiotic susceptibility testing of the bacteria contaminating fresh juices: Antibiotic susceptibility testing was performed according to the Kirby Power disc diffusion method. The results showed that, fecal *E. coli* demonstrated complete resistance to antibiotic Clarithromycin (100%), whereas it was less resistant to Sulfamethoxazole-Trimethoprim (3.85%) and sensitive to the rest of tested antibiotics including Ciprofloxacin, Imipenem and Oxacillin, Cefixime and Doxycycline and Cefuroxime (100%), whereas Sulfamethoxazole - trimethoprim (96.15%), followed by Amikacin (84.6%) and Amoxicillin, Cephalexin (76.9%). *E. coli* and *E. aerogenes* showed resistance to Clarithromycin and Amoxicillin (100%); alone *E. coli* recorded a partial resistant to Amikacin (89%), whereas *E. aerogenes* showed a complete resistance to the same antibiotic (100%). In contrast, the two isolates showed sensitivity to rest of antibiotics including Ciprofloxacin, Imipenem and Oxacillin, Cefixime and Doxycycline, Sulfamethoxazole-trimethoprim Cefuroxime and Cephalexin with percentage of sensitivity reached to (100%). *K. pneumoniae* showed resistance to Clarithromycin and Amoxicillin, Sulfamethoxazole - trimethoprim (95%), however, the isolate showed sensitivity to rest antibiotics including Ciprofloxacin, Cefixime, Doxycycline, Cefuroxime, and Cephalexin (100%), Amikacin (95%), Imipenem and Oxacillin (90%). *P. aeruginosa* has recorded resistance to Imipenem, Amikacin and Amoxicillin (100%). In contrast, it showed sensitivity to rest tested antibiotics including Ciprofloxacin, Cefixime and Doxycycline, Clarithromycin, Cefuroxime, and Cephalexin, Sulfamethoxazole trimethoprim (100%). *Staphylococcus schleiferi* showed resistance to Ciprofloxacin and Clarithromycin, Amikacin, Amoxicillin, Oxacillin, Cefixime and Cephalexin (100%), however, the contaminant showed a partial sensitivity to Sulfamethoxazole -trimethoprim and Imipenem (50%) and complete sensitive to the other tested antibiotics including Doxycycline, Cefuroxime (100%). *Staphylococcus aureus* showed full resistance to the most tested antibiotics (100%) with the exception of Imipenem and Sulfamethoxazole-trimethoprim, Doxycycline, Cefuroxime showed (50%) of resistance, table (3).

DISCUSSION

Drinking fresh juices are popular with people all times especially in summer. We often find juice street sellers. People drink these types of juices that overlook microbiology as well as the health standard, therefore high load of distributors and lack of hygienic practicing, so people often get sick. In this study, two main types of juice are used from different area in the city of Benghazi. Most juice samples showed high level of bacterial contamination. The resources of the contamination may be due to contaminated water or the use of ice to dilute juices.

It could be because contamination by unsterilized container, place, air, and preparing bare, hands. Microorganisms can spoil or decompose fruits through damaged surfaces, such as holes, cuts and cracks that occur during growth or harvesting (Mahale et al., 2008). Contamination from raw materials and equipment, additional processing conditions, improper handling, and the spread of unsanitary conditions contribute significantly to the entry of bacterial pathogens into juices prepared from these fruits (Nicolas et al., 2007; Ogodo et al., 2016; Oliveira et al., 2006).This study has revealed that, the most predominant microbes that contaminated the studied juices were *K. pneumoniae* and fecal *E. coli*, according to another study performed by (Fatema et al., 2016)on the microbiological quality assessment of hand-made juice in Dhaka City Street, where they also found that *E. coli* and *K. pneumoniae* have represented the most frequent isolated pathogens. A similar study was performed by (Berhanu et al., 2020) on the microbial quality spectrum of fresh and packaged fruit juices (pineapple and mango) Juices sold in supermarkets and cafes in Gondar city, Northwest Ethiopia, they have showed that the

bacteria that were the source of juice contamination were included *Salmonella sp.*, *Shigella sp.*, *E. coli*, *Pseudomonas sp*., *Klebsiella sp*. and *S. aureus*; whereas (Wedajo & Kadire, 2019) found less frequent bacterial diversity, the juice contamination included just of *Salmonella sp*., *E. coli*, and *S. aureus*. Plate count technique was performed in order to determine the most probable number (MPN) and to estimate the actual bacterial count (cfu/ml) into the testes juices as a food poisoning risk factor probability. According to the standard juice microbial quality, the contamination with fecal *E. coli* was max higher than exceeded limit (0cfu/ 10 ml and 100 ml) of sample, our results showed average contamination of fecal *E. coli* $1.23x10^5$ cfu/ 100 ml of juice, *E. coli* $3.7x10^4$ cfu/ 100 ml, *E. aerogenes* $5.74x10^4$ cfu/ 100 ml, *K. pneumoniae* $9.70x10^4$ cfu/100 ml. The contamination with *Staphylococcus sp.* was within the acceptable bacterial limitation count (less than $5x10^3$cfu/10 ml) compare to the Recommended Gulf Standard. Other similar results obtained by (Reddy et al., 2009), they have shown in the first bacterial isolation from fresh juices a level of bacterial contamination higher than our results even to the acceptable bacterial count, where the coliforms count reached to $1.4x10^5$ cfu/ 100 ml. In contrast, (Babiye, 2017) performed another study demonstrated that, the studied fresh mango and avocado juice samples were contaminated with 150 and 120 coliforms per 100 ml, this is still within the unacceptable bacterial count and probably this conflict with the level of hygiene safety during the juice preparation. (Reda et al., 2017) reported that the presence of thermotolerant fecal *coliform* can be attributed to fecal contamination of the water used to wash utensils, fruits, or transferred directly from the vendors, as well as the environment in which the juice is prepared, and leaving food at room temperature, at this case of these juices, and these can multiply to reach high concentrations, (Andrés et al., 2004) noticed that presence of coliform in fruit juice is not permitted by safe food consumption standard. This result is also in agreement with some other research works performed by (Ahmed et al., 2010; Mahale et al., 2008), where they showed the studied fruit juices were heavily contaminated by *E. coli.* A few reports have shown the prevalence of *staphylococci* in fruit

juice samples (Ahmed et al., 2010; Tambekar et al., 2009) about our study, *staphylococci* we isolated seven samples. Consequently confirmed the presence of *E. coli* and *Salmonella sp*. (Koneman & Allen, 2008) indicative of recent fecal contamination and unsanitary processing (Maturin & Peeler, 2001). An important task of the diagnostic microbiology is the performance of antimicrobial susceptibility testing in order to detect of the significant bacterial isolates that accumulate to the drug resistance pattern.

The results of the antimicrobial susceptibility tests revealed that all the Gram-negative pathogens were resistant to Clarithromycin, Amoxicillin, and Amikacin. For Gram *Staphylococcus spp.* showed the high rate of resistance to Ciprofloxacin, Clarithromycin, Amikacin, Amoxicillin, Oxacillin, Cefixime, and Cephalexin. (Uddin et al., 2017) in report on microbial safety of fruit juices, results from antibiogram test of the isolated microorganisms were *Klebsiella sp*. found to be less sensitive against Sulfamethoxazole -trimethoprim and Ciprofloxacin having 10% and 22% sensitivity, respectively whereas highest susceptibility was found against Nalidixic acid that was 90%. Most potent fecal *coliform*, *E. coli* showed moderate level of sensitivity against Sulfamethoxazole trimethoprim (55%). Additionally, Ampicillin was found to be less effective against *E. coli* as it showed about 95% resistance against it. Pathogenic *Staphylococcus sp.* showed highest resistance against Netilmicin (90%) followed by Ampicillin (84%). Antibiotic sensitivity of *K. pneumoniae* and *S. aureus* were found against Ciprofloxacin, Imipenem, gentamicin, levofloxacin with the exception of Amoxicillin (Sultana et al., 2019).

Table (1): Frequency of bacterial growth according to the areas

Geo-graphic areas	Areas of col-lection	Frist isolation				Second isolation			
		Growth		No Growth		Growth		No Growth	
		No. of collection points	% of growth	No. of collection points	% of no growth	No. of collection points	% of growth	No. of collection points	% of no growth
North	N1	4	100	0	0	4	100	0	0
	N2	4	100	0	0	4	100	0	0
	N3	3	75	1	25	4	100	0	0
	N4	4	100	0	0	4	100	0	0
Total	4	15	93.75	1	6.25	16	100	0	0
South	S1	3	75	1	25	4	100	0	0
	S2	4	100	0	0	1	25	3	75
	S3	4	100	0	0	4	100	0	0
	S4	4	100	0	0	4	100	0	0
Total	4	15	93.75	1	6.25	13	81.25	3	18.75
East	E1	4	100	0	0	4	100	0	0
	E2	4	100	0	0	4	100	0	0
	E3	3	75	1	25	4	100	0	0
	E4	3	75	1	25	4	100	0	0
Total	4	14	87.50	2	12.50	16	100	0	0
Center	C1	4	100	0	0	4	100	0	0
	C2	4	100	0	0	3	75	1	25
	C3	3	75	1	25	4	100	0	0
	C4	3	75	1	25	3	75	1	25
Total	4	14	87.50	2	12.50	14	87.50	2	12.50
Sum	16	58	90.62	6	9.37	59	92.19	5	7.81

Table (2): Diversity of the bacterial growth at different selected cafes and restaurants in the four studied areas

Area	fecal *E. coli*		*E. coli*		*E. aerogenes*		*K. pneumoniae*		*P. aeruginosa*		*S. schleiferi*		*S. aureus*	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
N1	4	50	1	12.5	0	0	2	25	1	12.5	0	0	0	0
N2	2	25	2	25	1	12.5	0	0	0	0	0	0	2	25
N3	3	37.5	0	0	0	0	5	62.5	0	0	0	0	0	0
N4	3	37.5	1	12.5	1	12.5	1	12.5	0	0	1	12.5	1	12.5
Total	12	37.5	4	12.5	2	6.25	8	25	1	3.13	1	3.13	3	9.37
S1	2	25	0	0	0	0	2	25	3	37.5	0	0	0	0
S2	3	37.5	0	0	1	12.5	1	12.5	0	0	0	0	0	0
S3	1	12.5	0	0	1	12.5	3	37.5	3	37.5	0	0	0	0
S4	0	0	1	12.5	0	0	3	37.5	4	50	0	0	0	0
Total	6	18.75	1	3.13	2	6.25	9	28.13	10	31.25	0	0	0	0
E1	5	62.5	1	12.5	1	12.5	1	12.5	0	0	0	0	0	0
E2	2	25	0	0	0	0	4	50	1	12.5	0	0	1	12.5
E3	3	37.5	2	25	1	12.5	1	12.5	0	0	0	0	0	0
E4	2	25	0	0	0	0	3	37.5	1	12.5	0	0	1	12.5
Total	12	37.5	3	9.38	2	6.25	9	28.13	2	6.25	0	0	2	6.25
C1	2	25	1	12.5	0	0	3	37.5	2	25	0	0	0	0
C2	3	37.5	0	0	1	12.5	3	37.5	0	0	0	0	0	0
C3	1	12.5	0	0	0	0	5	62.5	0	0	1	12.5	0	0
C4	0	0	0	0	1	12.5	5	62.5	0	0	0	0	0	0
Total	6	18.75	1	3.15	2	6.25	16	50	2	6.25	1	3.13	0	0
Sum	36	30.76	9	7.69	8	6.84	42	35.89	15	12.82	2	1.71	5	4.27

Table: (3) Characterization of Antibiotic sensitivity to the isolated bacteria contaminating the natural fresh juices

Antibiotics *vs* Bacteria	fecal *E. coli*		*E. coli*		*E. aerogenes*		*K. pneumoniae*		*P. aeruginosa*		*S. schleiferi*		*S. aureus*	
	R	S	R	S	R	S	R	S	R	S	R	S	R	S)
	(N) %	(N) %	(N) %	(N) %	(N) %	(N) %	(N) %	(N) %	(N) %	(N) %	(N) %	(N) %	(N) %	(N) %
CIP	(0)0	100	(0)0	(9)100	(0)0	(8)100	(0)0	(42)100	(0)0	(15)100	(2)100	(0)0	(5)100	(0)0
CLA	(36)100	(0)0	(9)100	(0)0	(8)100	(0)0	(40)95.2	(2)4.8	(0)0	(15)100	(2)100	(0)0	(5)100	(0)0
IMP	(0)0	100	(0)0	(9)100	(0)0	(8)100	(4)9.5	(38)90.5	(15)100	(0)0	(1)50	(1)50	(0)0	(5)100
AK	(5)15.4	(31)84.6	(8)88.8	(1)11.2	(8)100	(0)0	(2)4.8	(40)95.2	(15)100	(0)0	(2)100	(0)0	(5)100	(0)0
AX	(8)23.1	(28)76.9	(9)100	(0)0	(8)100	(0)0	(40)95.2	(2)4.8	(15)100	(0)0	(2)100	(0)0	(5)100	(0)0
OX	(0)0	(36)100	(0)0	(9)100	-	-	(0)0	(42)100	-	-	(2)100	(0)0	(5)100	(0)0
CFX	(0)0	(36)100	(0)0	(9)100	(0)0	(8)100	(4)9.5	(38)90.5	(0)0	(15)100	(2)100	(0)0	(5)100	(0)0
CL	(8)23.1	(28)76.9	(0)0	(9)100	(0)0	(8)100	(0)0	(42)100	(0)0	(15)100	(2)100	(0)0	(5)100	(0)0
SXT	(1)3.9	(35)96.1	(0)0	(9)100	(0)0	(8)100	(40)95.2	(2)4.8	(0)0	(15)100	(1)50	(1)50	(0)0	(5)100
DO	(0)0	(36)100	(0)0	(9)100	(0)0	(8)100	(0)0	(42)100	(0)0	(15)100	(0)0	(2)100	(0)0	(5)100
CXM	(0)0	(36)100	(0)0	(9)100	(0)0	(8)100	(0)0	(42)100	(0)0	(15)100	(0)0	(2)100	(0)0	(5)100

CONCLUSION

This study has concluded that, most of the studied fresh juice samples showed bacterial growth, interestingly, this is revealed increase the bacterial contamination that indicating risk factor leading to possibility of illness, therefore, control microbial contamination through the application of the restricted hygiene strategies which needed to be considered in order to provide the markets with safe fresh juice products. Increase the educational awareness on the microbial hazards for the people who working on the juice preparations including food safety and sanitization which assist them to gain responsibility for quality control. Routine microbiological evaluation gives enough information on the expected microbial risk factors.

Duality of interest: The authors declare that they have no duality of interest associated with this manuscript.

Author contributions: Dr. Bozakouk developed the theoretical formalism, Mrs BaLshikh performed the analytic calculations and performed the numerical simulations. Both Dr Bozakouk, Mrs BaLshikh and Dr. Bumadian contributed to the final version of the manuscript. Dr. Bozakouk supervised the project.

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Synthesis of Plasma-Polymerized Toluene Coatings by Microwave Discharge

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Abstract: Plasma- polymerized coatings were successfully applied on aluminum alloy, AA2024, surface for corrosion protection. The plasma polymerization process was carried out by low pressure microwave plasma at room temperature. The effect of microwave plasma power on the corrosion resistance of polymer coatings was investigated using the potentiodynamic polarization technique. As the microwave plasma power increased, the relative protective efficiency increased. Polymer coatings on alloy surfaces suppressed both anodic and cathodic reactions. The increment in protective efficiency was due to a higher degree of cross-linking in the coating. These findings suggest that the toluene polymer coatings provide a considerable protection barrier for aluminum alloys.

تصنيع طلاءات التولوين المبلمرة بالبلازما بواسطة التفريغ الكهربائي بالموجات الدقيقة

الكلم ـات المفتاحي ـة :
الحماية من التآكل،
الترسـ ـيب الكيميـ ـائي المعزَّز بالبلازما،
طلاءات التولوين.

المستخلص : قام الباحث بتطبيق الطلاءات المبلمرة بالبلازما بنجاح على سطح سبائك الألومنيوم (AA2024) لغرض الحماية من التآكل. ونُفِّذت عملية بلمرة البلازما بواسطة بلازما الموجات الدقيقة منخفضة الضغط عند درجة حرارة الغرفة. ولدراسة تأثير قدرة البلازما على مقاومة التآكل لطلاءات البوليمر استخدم الباحث تقنية الاستقطاب الديناميكي الفعال. ولوحظ أنه مع زيادة طاقة البلازمـ ـا ازدادت كفـ ـاءة الحمايـ ـة النسـ ـبية لهـ ـذه الطلاءات. وجود الطلاءات البوليمرية على أسطح السبائك أدَّت إلى كبت التفـ ـاعلات الأنوديـ ـة والكاثوديـ ـة. وجاءت الزيادة في كفاءة الحماية نتيجة ارتفاع درجة التشابك المتبادل لسلاسل البوليمر داخل هذه الطـ ـلاءات. تشير هذه النتائج إلى أن طلاء بوليمر التولوين يوفر حاجز حماية كبير من التآكل لسبائك الألومنيوم.

INTRODUCTION

Aluminum and its alloys have excellent mechanical and physical properties for several applications. Because of the unique combination of high strength-to-weight ratio, makes it an ideal material for aerospace applications where weight reduction is considered (Abd El-Hameed & Abdel-Aziz, 2021). The aluminum alloy (AA2024) is one of the most important groups of aluminum alloys that widely used in satellites and space structures(Zuo et al., 2015). Aluminum alloys, on the other hand, are more susceptible to corrosion. This could limit its usage in aerospace industries, where harsh service environments are unavoidable. The most straightforward way to prevent corrosion is to coat the alloy surface to keep it from coming into contact with the environment. For protection, the surface is clad with Al-1Zn, which may reduce fatigue strength(Navas et al., 2015). In addition, Chromate conversion coatingsare commonly applied to aluminum alloy surface. However, the major concern with this coating is its toxicity(Yan et al., 2009). Polymer coatings

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for corrosion protection of metallic surfaces and their alloys have attracted considerable interest in the fields of research and industry(Ates, 2016; Olajire, 2018). Among recent techniques used to deposit polymer coatings on material surfaces, plasma enhanced chemical vapor deposition (PECVD) (Ardic & Gifvars, 2017; Bowen & Cheneler, 2019; Fermi et al., 2019; Krtouš et al., 2021; Mitev et al., 2016; Zhou et al., 2020). It is a unique technique for fabricating corrosion-resistant polymer coating from a variety of organic materials(Aramaki, 1999; Esbayou et al., 2018; Grundmeier et al., 2003; Jaritz et al., 2019; Natishan et al., 1995; Singh-Beemat et al., 2013; Wang et al., 1996). The obtained coatings are pinhole-free and highly cross-linked(Martin, 2009). In the current research, a polymer toluene coating was successfully deposited on an aluminum alloy substrate(AA2024) with good corrosion abilities.

The effect of microwave plasma power on the corrosion resistance of polymerized toluene coatings, prepared by the PECVD method, was studied systematically. Microwave plasma with high frequencies (2.45 GHz) was used to generate a glow discharge to initiate the plasma polymerization process.

MATERIALS AND METHODS

Polymer coatings were prepared using plasma-enhanced chemical vapor deposition (PECVD) method. Figure 1 shows a schematic diagram of the low-pressure plasma reactor used for PECVD. Plasma polymerization was carried out in a stainless steel vacuum chamber. The chamber was evacuated from atmospheric air to 10^{-5}Torr. Microwave plasma (0-900 W-2.45 GHz) was used to generate a glow discharge necessary to initiate the polymerization process. Aluminum alloy (AA2024), with dimensions of (1.5×1.5) cm^2, were used to deposit the polymer coatings.

The coating quality is directly impacted by the substrate conditions. To achieve a homogeneous, defect-free deposition, the substrates were cleaned using distilled water and acetone. Then they were exposed to Ar plasma in situ to create an oxygen-free surface and improve film adhesion. This process was carried out at 720 watt of Mw power and lasted up to 20 min. Toluene monomer was used as an organic precursor. The PECVD process was run at 100-800 watt, a deposition time of 10-25 minutes, and a toluene/argon ratio of 15%. A spectrometer analyzer was used to characterize the chemical composition of aluminum alloy. Potentiodynamic polarization measurements were carried out in a 3.5 wt.% NaCl solution at room temperature. Aluminum samples, both bare and coated with toluene coating, were connected with an isolated electric wire and all surfaces were painted with Lacquer 45, leaving only 1 cm^2 exposed to the electrolyte. The electrodes were immersed in the solution for 3 hours.The electrode's potential was then swept at 0.166 mV/s from a starting potential of -400 mV vs. E_{corr} to a final potential of 1000 mV vs. E_{corr}. The data acquisition and data analysis were performed using the ACM instrument's software (GILLAC-UK).

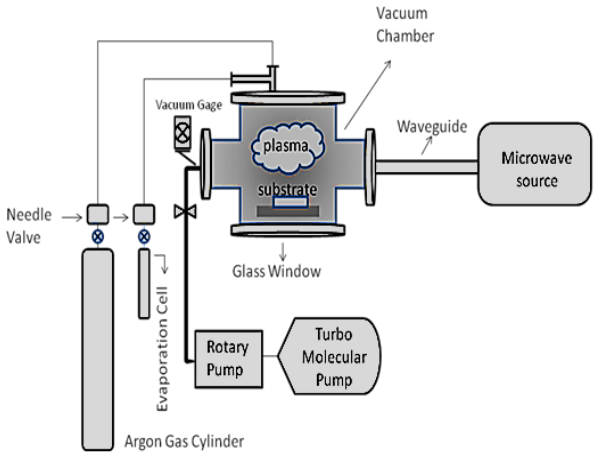

Figure: (1). A schematic diagram of micro wave plasma reactor

RESULTS AND DISCUSSION

The chemical analysis of aluminum alloy, AA2024, is shown in Table 1. A toluene coating was deposited on aluminum alloy surface using low-pressure microwave plasma. All coatings were subjected to the same

plasma treatment time of 25 min at room temperature. Ji et al.(Ji et al., 2013)prepared toluene coatings on the aluminum substrate using RF discharge plasma. They reported that the increase in treatment time beyond 30 min led to the formation of a brown powder on the aluminum surface.

Table:(1). Chemical analysis of aluminum alloy (AA2024)

Element	Al	Cu	Si	Fe	Mn	Mg	Cr	Zn	Ti
Wt %	90	3.8	0.5	0.5	0.3	1.6	0.1	0.25	0.15

Figure 2 shows the typical potentiodynamic polarization behavior of as received aluminum alloy, AA2024, in a 3.5 wt. % NaCl solution. In the potential range investigated, the polarization curve displays three unique regions: the active (Tafel) zone, the active-passive transition region, and the limiting current region. The protective efficiency (Pi) and corrosion rate can be derived from the potentiodynamic polarization curve. The following formula was used to calculate the protective efficiency (Pi) of the coating(Enos & Scribner, 1997).

Protective efficiency (Pi)=100 x $(1-i_{corr} / i°_{corr})$ (1)

Where i_{corr} and $i°_{corr}$ represent the corrosion current densities with and without the deposited coatings, respectively. The values were obtained by extrapolating the cathodic Tafel lines of the anodic and cathodic branches of the potentiodynamic curve(Aramaki, 1999; Nozawa & Aramaki, 1999; Tsuji et al., 2000).The slope of the cathodic and anodic branches of the polarization curve determines the anodic and cathodic reactions at the metal/coating interface. Higher slope values are associated withlower anodic and cathodic reaction velocity (Singh-Beemat et al., 2013).The corrosion rate was calculated using the following equation:

$$\text{Corrosion Rate} = (w \times A) / \rho \quad (2)$$

Where w is the mass of material removed, A is the exposed surface area and ρ is the material density. Table 2 shows the corrosion properties of AA2024 substrate before coated with toluene coating.

Table:(2). Corrosion properties of as received AA 2024 obtained from potentiodynamic curve

E_{corr} (mV)	i^0_{corr} (µA / cm^2)	corrosionrate (mm/year)
-747	11.65±0.05	0.13

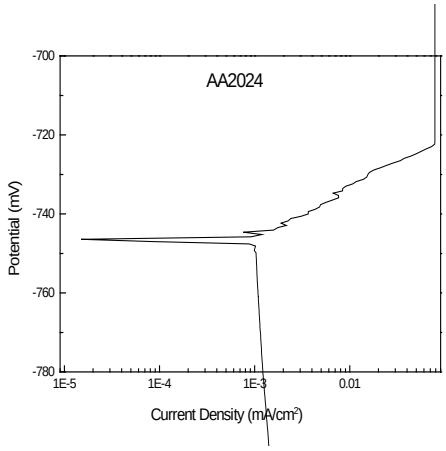

Figure: (2). Potentiodynamic curve of as received aluminum alloy, AA2024, in 3.5 wt.% NaCl solution

The $i°_{corr}$ value of the bare sample increased due to the presence of Cl^{-1} ions in the immersion solution, resulting in severe localized corrosion on the sample surface. Similar results have been reported for sol-gel Ceria coatings on the surface of AA2024 alloy(Zuo et al., 2015).Figure 3 shows the corrosion behavior of the aluminum alloy substrates covered with toluene coatings at different microwave powers. Table 3 summarizes the data obtained from the analysis

of each potentiodynamic curve. It clearly demonstrates that the coated samples exhibit a marked improvement in the protective abilities compared to the uncoated one. The I_{corr} values of the coated samples were relatively lower, compared to as received AA2024 alloy. Both the cathodic and anodic branches of the polarization curves were remarkably suppressed by coverage of the electrodes with the toluene coating. Yu et al. have reported that the densely packed toluene films on copper substrate can inhibit for the copper dissolution caused by diffusion of chloride ions to the copper surface(Yu et al., 2003).

Table:(3). Corrosion properties of aluminum electrodes covered with toluene coatings with different m.w. Powers at 15% toluene ratio

Power (watt)	E_{corr} (mV)	i_{corr}(±0.05 µA/cm^2)	P_i%	Corrosion Rate (mm/year)
720	-850	4.20	65.00	0.040
540	-737	4.30	63.00	0.047
360	-742	5.10	57.50	0.053

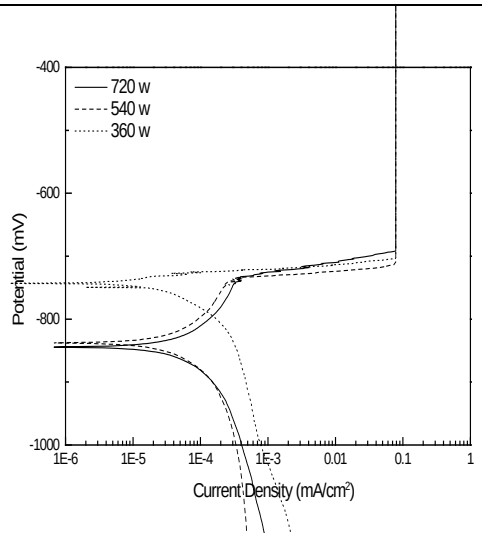

Figure: (3). Polarization curves of AA2024 coated with toluene films as a function of m.w. Power

With an increase in microwave power, the protective abilities of toluene coatings improved slightly. Highest protective efficiency of 65% was obtained at 720 watt. At a power of less than 360 w, the deposition process could not take place. Joo et al.(Joo et al., 2000) studied the Effects of plasma power on the properties of low-k polymer-like organic thin films deposited by PECVD method using the toluene as the precursor. Their results showed that when the plasma power is low enough, it cannot decompose the benzene ring in the toluene precursor. Yu et al.(Yu et al., 2003) reported that the protective abilities of toluene films, prepared by the PECVD method, increased with increasing RF power.

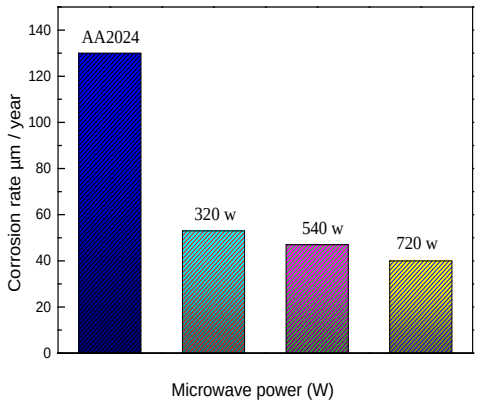

Figure: (4). Corrosion rate of aluminum alloy electrodes as a function of microwave power

Figure4 shows the corrosion rate calculated from Table 2 and Table 3. As shown in Figure4, the corrosion rate decreased in the presence of toluene coating. The coatings deposited at a 15% - 720 W showed an obviousreduction in its corrosion rate value, 40µm/year. This improvement in corrosion resistance was attributed totoluene coatings that inhibit both anodic and cathodic reactions. The toluene coatings were densely packed and firmly linked, which suppressed oxygen reduction and diffusion of an electrolyte onto the alloy surface. The presence of toluene coatings could significantly reduce aluminum's susceptibility to environmental corrosion.

CONCLUSION

Plasma polymerized organic coatings were successfully prepared using plasma enhanced chemical vapor deposition method.

The protective abilities of organic coatings as a function of microwave power were characterized by potentiodynamic polarization test. The presence of organic coatings suppressed oxygen reduction and diffusion of an electrolyte onto the alloy surface. The protective efficiency of the toluene coatings increased with increasing microwave power. The highest protective efficiency was 65% at 720 watts of microwave power. Toluene coatings with increasing microwave power had the higher degree of cross-linking, suggesting that better corrosion protection for AA2024 aluminum alloy.

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Author contributions: The author confirms sole responsibility for the following: study conception and design, data collection, analysis and interpretation of the results, and manuscript preparation.

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Antimicrobial Susceptibility Patterns of *Escherichia coli* from Urine Isolates

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Abstract: Urinary tract infections (UTIs) are predominantly caused by *Escherichia coli* (*E. coli)*. Increasing *E. coli* resistance to antibiotics is a major concern worldwide. Since UTIs are often treated by trial and error, measuring antimicrobial resistance (AMR) is important. However, there isn't much information about the rate of antimicrobial resistance to *E. coli* in the Libyan community. To determine rate of antimicrobial susceptibility patterns of *E. coli* urine isolates, in Al-Bayda, Libya. A retrospective study, in which 104 *E. coli* urine isolates were conducted using the antimicrobial susceptibility profile (antibiogram) of six different antibiotics against *E. coli*, isolates, were collected from several medical laboratories. Out of the 104 *E. coli* urine isolates, the MDR was 39.4%. The overall frequency of isolates resistant to ceftriaxone was 62.5%, trimethoprim-sulfamethoxazole (TMP-SMZ)(54.8%), Amoxicillin-Clavulanic acid (47.11%), ciprofloxacin (26%), nitrofurantoin (18.26%), and levofloxacin (15.4%). Prevalence of AMR among Libyan outpatient urine-isolated *E. coli* was high, with a high incidence of multidrug-resistance. The knowledge of antibiotic resistance rates in the region helps inform empiric treatment of community-onset UTI and highlights the antibiotic resistance profile to clinicians.

أنماط حساسية مضادات الميكروبات لعزلات بول الإشريكية القولونية

الكلمات المفتاحية :
ع . دوى المس . الك البولية؛
مقاوم .ة مض .ادات الميكروبات؛
الإشريكية القولونية؛
الأنتيبيوجرام (تحسس الجراثيم للمضادات).

المستخلص : إن التهابات المسالك البولية تسببها في الغالب الإشريكية القولونية، زيادة مقاومة الإشريكية القولونية للمضادات الحيوية هو مصدر قلق كبير في جميع أنحاء العالم، نظرًا إلى أن عدوى المسالك البولية تُعالَج غالبًا عن طريق التجربة واحتمالية الخطأ، فمن المهم قياس مقاومة مضادات الميكروبات؛ بما أنه لا تتوافر الكثير من المعلومات حول معدل مقاومة مضادات الميكروبات للإشريكية القولونية في عدوى المسالك البولية التي تظهر في المجتمع الليبي. تم تحديد تواتر وأنماط الحساسية لمضادات الميكروبات لعينات الإشريكية القولونية من عينات البول خلال جائحة فيروس كورونا في مدينة البيضاء، ليبيا. حيث أُجريت دراسة بأثر رجعي لـ 104 من عزلات الإشريكية القولونية المعزولة من البول جُمعت من عدة مختبرات طبية وذلك باستخدام بيانات التحسُّس لمضادات الميكروبات (الأنتيبيوجرام) لستة مضادات حيوية مختلفة. حيث أظهرت النتائج أنه من بين 104 عزلة من الإشريكية القولونية، كان معدل المقاومة المتعددة للأدوية 39.4٪. وكان الإجمالي للعزلات المقاومة للإشريكية القولونية على النحو الآتي: للسيفترياكسون 62.5٪، تريميثوبريم–سلفاميثوكسازول (54.8٪)، حمض أموكسيسيلين–كلافولانيك (47.11)، سيبروفلوكساسين (26٪)، نتروفورانتوين (18.26٪) و ليفوفلوكساسين (15.4٪). حيث كانت للمضادات الحيوية الآتية أعلى مقاومة لمضادات الميكروبات: سيفترياكسون، سلفاميثوكسازول / تريميثوبريم، أموكسيسيلين + حمض الكلافولانيك، وسيبروفلوكساسين، بهذا الترتيب. على الرغم من أن الليفوفلوكساسين والنيتروفورانتوين كانا أكثر المضادات الحيوية حساسية، فإن العمر عامل كبير في مدى حساسية عزلات بكتريا القولونية تجاه المضادات الحيوية.

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INTRODUCTION

Urinary tract infections (UTIs) are considered the most common community-acquired and nosocomial infections. It has been reported that 150 million cases of UTI occur each year worldwide (Medina & Castillo-Pino, 2019). The prevalence of UTI varies with age and gender. About 40–60% of women will get an UTI in their lifetime, which is more than the 12% of men who will get one (Kot, 2019; Medina & Castillo-Pino, 2019). It is the second most common infection after respiratory tract infections (Elsayah et al., 2017).

E. coli accounts for up to 80% of isolated bacteria causing UTIs (Niranjan & Malini, 2014; van Driel et al., 2019). Additionally, *E. coli* is also capable of infecting the lungs, surgical sites, bloodstream, and meninges (Forsyth et al., 2018). A local study performed by (Ismail et al. 2018) in Eastern Libya to determine the incidence of UTIs found that the most prevalent uropathogen was *E. coli* (48%). Another national study enrolled 1,790 patients with UTIs, *E. coli* was the predominant uropathogen, being isolated at 55.8% (Abujnah et al., 2015). Amongst bacteria detected in 2209 urine specimens from patients with UTI in Tripoli, Libya, *E. coli* was the major positive isolate (24%) followed by Staphylococcus spp. (8%) (Ghenghesh et al., 2003).Uncomplicated UTIs are commonly treated by empirical antibiotics without prior antibiotic susceptibility testing. These include: Nitrofurantoin, fosfomycin, trometamol, and TMP-SMZ are recommended as first-line therapy for uncomplicated cystitis. Amoxicillin-clavulanic acid is recommended as first line-therapy for mild and moderate pyelonephritis or complicated UTI, as well as alternative empiric therapy for uncomplicated UTIs. In the treatment of uncomplicated cystitis, ciprofloxacin should not be considered as a first-line antibiotic, but as an alternative (Kot, 2019).

Many studies have reported that antimicrobial resistance in *E. coli* has been increasingly observed and reported worldwide due to several factors, such as the use of empirical antibiotics to treat UTIs without antibiotic sensitivity testing as the international protocols recommend (Kot, 2019; Shuaib et al., 2021). The development of multidrug resistance (MDR), which is the resistance to one or more classes of antimicrobials against *E. coli* strains, has caused increasing concern over the empirical treatment options in the case of UTIs with *E. coli*. Fluoroquinolones (levofloxacin and ciprofloxacin), cephalosporins (Cefixime and Ceftriaxone), sulfonamides (TMP-SMX), penicillin (Amoxicillin-Clavulanic acid), and nitrofurantoin are among the MDR classes reported (Abduzaimovic et al., 2016). During the COVID-19 pandemic in May 2021, a WHO report shows worrying trends, especially in low- and middle-income countries like Libya, where more reports are being sent to the Global Antimicrobial Resistance and Use Surveillance System (GLASS), which was the first global effort to standardize AMR surveillance (WHO, 2021).

The WHO reported over 3 million laboratory-confirmed bacterial infections resistant to WHO priority list pathogens in 70 countries. Although it is too early to link the higher resistance rates to the COVID-19 pandemic. Resistance rates have increased six-fold since sites began sharing AMR surveillance data in 2017 (WHO, 2020, 2021). Prior to the COVID-19 epidemic, the Arab world had already experienced alarming levels of AMR (Dandachi et al., 2019) Recognizing the scarcity of research on the topic, this study studied the impact of COVID-19 on *E.coli* AMR and antimicrobial stewardship (AMS) in Libya as an Arab League country .

This study aimed to determine the prevalence of antimicrobial susceptibility profile (Antibiogram) of *E. coli* isolates collected from urine samples of outpatients suffering from UTIs.

MATERIALS AND METHODS

Study design and participants: This study was a retrospective study conducted in the medical laboratories at Al-Bayda, Libya, from January 2021 to April 2022. Outpatients with UTI infections caused by *E.coli* of all ages and both sexes were tested for sensitivity to 5 antibiotics. Excluding incomplete patient records, comorbid cases, and cases with a recurrent history of UTIs.

Sample size, and collection techniques
The sample size was calculated using the Epi-info software program to be 104 urine samples. The urine specimens and their age and sex were labeled.

Urine culture; The standard loop method, which is semi-quantitative, was used to cultivate urine. Standard procedures, such as gram stain, blood agar, MacConkey agar, and API (Analytical Profile Index) 20E, were used to identify isolated *E. coli*

Procedures: The antibiotic sensitivity test was done on Mueller-Hinton agar by the Kirby-Bauer disc diffusion test as per Clinical and Laboratory Standard Institute (CLSI) guidelines (Clinical & Institute, 2012). The following antimicrobial agents were tested for their resistance and susceptibility: amoxicillin/clavulanic acid (30 µg), TMP/SXT (25 µg), ciprofloxacin (5 µg), nitrofurantoin (300 µg), Levofloxacin (5 µg) and ceftriaxone (30 µg). An isolate was considered an MDR if it was found resistant to three or more antimicrobial classes belonging to different classes/groups of antimicrobials.

Statistical Analysis: The data was analyzed using SPSS version 25 (IBM Corp., Armonk, NY, USA). Differentials were considered statistically significant at $p< 0.05$. The qualitative and discrete sociodemographic variables were presented as frequency and percent. The Chi-square test was performed to test the relationship between sociodemographic factors and antibiotic resistance and susceptibility. The predictors of antibiotic resistance and susceptibility of *E. coli* isolates were identified using multinomial logistic regression analysis.

RESULTS

The majority of the studied samples were females 99 (95.5%). Out of the 104 *E. coli* urine isolates, Augmentin resistance was 49 (47.1%). The susceptibility patterns of *E. coli* strains were significantly ($p = 0.03$*) affected by the patient age, especially the age group 18–25 y. The OR (95% C.I] was 2.28 (-4.12-0.44) with $p = 0.02$* compared to participants ≥65 y (Table 1)**.**

ciprofloxacin resistance was 27 (26. 0%).There was a statistically significant relationship between it and the age ($p = 0.01$*), especially the age group 18–25 y, and ≥65 y. The OR (95% C.I] was 3.18(1.62-56.7) and 2.95(1.9-81.9) in order with $p <0.05$ compared to participants aged less than 18 y (Table 2).

Nitrofurantoin resistance was 19(18.3%). There was a statistically significant (P value less than 0.05) relationship between it and the age and sex of participants. Among males, the OR (95% C.I] was -2.05 (-3.92/-0.18), and had significantly lower resistance ($p = 0.03$). Compared to participants aged more than 65 years, nitrofurantoin resistance was significantly lower ($p<0.05$) among the age groups of 18–35 years and less than 10 years**.** As shown in (Table 3)**.**

Levofloxacin resistance was 16(15.4%). There was a statistically significant ($p< 0.05$) relationship between it and the age and sex of participants. Among males, the OR (95% C.I] was 2.29(4.18-0.41), and had significantly lower resistance ($p = 0.02$). Compared to participants aged more than 65 years, Levofloxacin resistance was significantly lower ($p<0.05$) among the age groups of 18–<25 years and less than 10 years. (Table 4).

Table (1). Background information, and Frequency, Determinants., and Predictors of Augmentin susceptibility patterns to *E. coli* strains urine isolates

Age groups (y)	Total T=104	Augmentin		X^2 (P)	Predictors of Augmentin resistance OR[95% C.I]	P
		Sensitive 55(52.9) F (%)	Resistance 49(47.1) F (%)			
1-<10	19(18.3)	11(20.0)	8(16.3)	15.1 (0.03*)	-1.29(-2.90-0.310)	0.11
10-<18	5(4.8)	4(7.3)	1(2.0)		-2.37(-4.93-0.19)	0.07
18-<25	14(13.5)	11(20.0)	3(6.1)		-2.28(-4.12-0.44)	0.02*
25-<35	32(30.8)	19(34.5)	13(26.5)		-1.36(-2.86-0.14)	0.08
35-<45	10(9.6)	3(5.5)	7(14.3)		-0.13(-2.02-1.76)	0.89
45-<65	13(12.8)	4(7.3)	9(18.4)		-0.17(-1.94-1.60)	0.85
≥65	11(10.6)	3(5.5)	8(16.3)		Reference	
Sex						
Male	5(4.8)	1(1.8)	4(8.2)	2.28 (0.13)	-1.6(0.02-1.93)	0.17
Female	99(95.2)	54(98.2)	45(91.8)			

*p <0.05 there was a statistical significant difference

Table (2). Patterns of ciprofloxacin susceptibility of isolated *E. coli* at different age groups

Age groups (y)	Ciprofloxacin		X^2 (P)	Predictors of ciprofloxacin resistance OR[95% C.I]	P
	Sensitive 77(74.0) F (%)	Resistance 27 (26.0) F (%)			
1-<10	17(22.1)	2(7.4)	18.1 (0.01*)	Reference	
10-<18	2(2.6)	3(11.1)		2.77(0.5-44.1)	0.11
18-<25	13(16.9)	1(3.7)		3.18(1.62-56.7)	0.02*
25-<35	26(33.8)	6(22.2)		0.21(0.07-21.6)	0.89
35-<45	7(9.1)	3(11.1)		1.30(0.41-33.5)	0.25
45-<65	7(9.1)	6(22.2)		2.62(1.38-55.9)	0.12
≥65	5(6.5)	6(22.2)		2.95(1.9-81.9)	0.03*
Sex					
Male	2(2.6)	3(11.1)	3.17 (0.08)	Reference	
Female	75(97.4)	24(88.9)		-1.54(0.03-1.35)	0.10

*p <0.05 there was a statistical significant difference

Table (3). Patterns of Nitrofurantoin susceptibility of isolated *E. coli* at different age groups

Age groups (y)	Nitrofurantoin		X^2 (P)	Predictors of Nitrofurantoin resistance OR[95% C.I]	P
	Sensitive 85(81.7) F (%)	Resistance 19 (18.3) F (%)			
1-<10	17(20.0)	2(10.6)	15.9 (0.04*)	-1.96(-3.84/-0.72)	0.04*
10-<18	4(4.7)	1(5.3)		-1.20(-3.69-1.29)	0.34
18-<25	14(16.5)	0(0.0)		-2.38(-4.74/-0.03)	0.047*
25-<35	27(31.8)	5(26.3)		-2.52(-4.38/-0.67)	0.008*
35-<45	6(7.1)	4(21.1)		-2.02(-4.39-0.37)	0.097
45-<65	11(12.9)	2(10.5)		-0.63(-2.30/1.04)	0.46
≥65	6(7.1)	5(26.3)		Reference	
Sex					
Male	2(2.4)	3(15.8)	6.12 (0.013*)	-2.05(-3.92/-0.18)	0.03*
Female	83(97.6)	16(84.2)		Reference	

*p <0.05 there was a statistical significant difference

Table 4; Patterns of Levofloxacin susceptibility of isolated *E. coli* at different age groups

Age groups (y)	Levofloxacin		X^2 (P)	Predictors of Levofloxacin resistance OR[95% C.I]	P
	Sensitive 88(84.6) F (%)	Resistance 16 (15.4) F (%)			
1-<10	17(19.3)	2(12.6)		-1.96(3.84- 0.07)	0.04*
10-<18	4(4.5)	1(6.3)		-1.20(3.69-1.29)	0.34
18-<25	13(14.8)	1(6.3)		-2.38(2.7-0.03)	0.047
25-<35	30(34.1)	2(12.5)	16.1	-2.53(4.38-0.67)	0.008*
35-<45	9(10.2)	1(6.3)	(0.024*)	-2.01(4.39-0.37)	0.097
45-<65	9(10.2)	4(25.0)		-0.63(2.30-1.04)	0.46
≥65	6(6.8)	5(31.3)		Reference	
Sex					
Male	2(2.3)	3(18.8)	8.03	-2.29(4.18-0.41)	0.02*
Female	86(97.7)	13(81.3)	(0.005*)	Reference	

Ceftriaxone resistance was 65(62.5 %). There was a statistically significant (p less than 0.05) relationship between Ceftriaxone resistance and the age of participants. Compared to participants aged more than 65 all age groups except the age group between 45 and less than 65y are significant (p<0.05) risky to Ceftriaxone resistance (Table 5).

TMP-SMZ resistance was (57,54.8%). There was a statistically significant (p less than 0.05) relationship between TMP-SMZ resistance and the age and sex of participants. Among females, the OR (95% C.I) was 17.2 (16.8/-17.9) and had significantly lower resistance (p = 0.00). Compared to participants aged more than 65 years, nitrofurantoin resistance was significantly lower (p<0.05) The OR (95% C.I] was 2.35 (4.429-0.42) among the age groups of 18–<25 years (Table 6).

Table (5). Patterns of ceftriaxone susceptibility of isolated *E. coli* at different age groups

Age groups (y)	Ceftriaxone		X^2 (P)	Predictors of ceftriaxone resistance OR[95% C.I]	P
	Sensitive 39(37.5) F(%)	Resistance 65 (62.5) F(%)			
1-<10	10(25.6)	9(13.8)		2.41(4.65-0.16)	0.035*
10-<18	4(10.3)	1(1.5)		3.69(6.69-0.68)	0.016*
18-<25	8(20.5)	6(9.2)		2.59(4.90-0.28)	0.028*
25-<35	13(33.3)	19(29.2)		1.92(4.09-0.25)	0.083
35-<45	3(7.7)	7(10.8)	22.12	1.45(3.92-1.001)	0.246
45-<65	0(0.0)	13(20.0)	(0.002*)	19.9(17.1-20.2)	0.03*
≥65	1(2.6)	10(15.4)		References	
Sex					
Male	0(0.0)	5(7.7)	3.15	References	
Female	39(100.0)	60(92.3)	(0.19)	17.1(16.1-18.9)	0.00*

*p <0.05 there was a statistical significant difference

Table: (6) Patterns of TMP-SMZ susceptibility of isolated *E. coli* at different age groups

	TMP-SMZ		X^2 (P)	Predictors of Septrin resistance OR[95% C.I]	P
	Sensitive 47(47.2) F (%)	Resistance 57 (54.8) F (%)			
Age groups (y)					
1-<10	4(8.5)	15(26.3)		0.76(0.88-2.41)	0.37
10-<18	2(4.3)	3(5.3)		0.15(2.23-2.02)	0.89
18-<25	12(25.5)	2(3.5)		2.35(4.29-0.42)	0.017*
25-<35	19(40.4)	13(22.8)		0.94(2.35-0.46)	0.194
35-<45	3(6.4)	7(12.3)	20.65 (0.004*)	0.29(1.53-2.12)	0.758
45-<65	3(6.4)	10(17.5)		0.64(1.13-2.43)	0.48
≥65	4(8.5)	7(12.3)		References	---
Sex					
Male	0(0.0)	5(8.8)	4.33	Reference	0.00*
Female	47(100.0)	52(91.2)	(0.04*)	-17.2(16.8/17.9)	

*p <0.05 there was a statistical significant difference

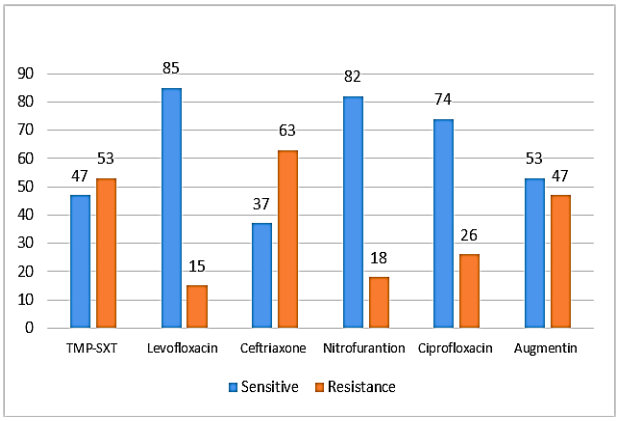

Figure (1). Antimicrobial susceptibility patterns of *E. coli* strains to different antimicrobial agents

All *E. coli* isolates (104) were tested for their susceptibility using a panel of antibiotics. The *E. coli* isolates were resistant mostly to the tested antibiotics as follows: Ceftriaxone (65,62.5%), TMP-SMZ (57/104; 54.8%), amoxicillin + clavulanic acid (49/104; 47.11%), and ciprofloxacin (27/104; 26.8%) were all found to have high rates of resistance. While the antibiotics of higher sensitivity were levofloxacin (88,84.6%), nitrofurantoin (85.81.7%).

DISCUSSION

Uropathogenic *Escherichia coli* (UPEC) is one of the main bacteria causing UTIs. The rates of UPEC with high resistance to antibiotics and MDR *E. coli* have increased dramatically in recent years and could complicate the treatment (Ramírez-Castillo et al., 2018),. This emergence of global concern poses a major challenge to physicians and public health worldwide (Paterson, 2000). The choice of empirical antimicrobial therapy is based on antibiogram patterns that show resistance trends among pathogens. Because antibiotic resistance patterns change over time, it is important for clinicians to learn about the information they have seen (Kumarasamy et al., 2010).

Limited studies show the resistance rate to antibiotics used in treating UTIs against *E. coli* in other areas and cities in Libya during the COVID-19 pandemic, studies carried out between 2002 and 2008 reported an increase in *E. coli* resistance rates to Ciprofloxacin and other fluoroquinolones (Ghenghesh et al., 2013). High rates of resistance to TMP-SMZ were also observed from 1990 to 1999 for *E. coli* from UTIs in Tripoli and Benghazi (Ghenghesh et al., 2013). AMR patterns of *E. coli* from patients with UTIs who attended Zawiya Teaching Hospital in Zawiya city between November 2012 and June 2013 were (37%), (23.1%) and (19.2%) for TMP-SMZ, Ciprofloxacin, and Levofloxacin. MDR (resistance to 3 antimicrobial groups) was

found in (33.2%) (Abujnah et al., 2015).

As regards the sex; According to the findings (95.2 %), between January 2021 and April 2022 in Al-Bayda, Libya, the *E. coli* urine isolates in outpatients were detected more frequently in women. These findings are in line with prior research (Deshpande et al., 2011; Haque et al., 2015; Keah et al., 2007). Furthermore, male patients' isolates had higher antimicrobial drug resistance than female patients' isolates, male infections may be more difficult to treat since male strains have higher rates of antibiotic resistance, which could lead to repeated infections. These findings were consistent with previous research (Ali et al., 2016; Tabasi et al., 2015; Wagenlehner et al., 2007). Isolates should be screened for antibiotic susceptibility before deciding on a treatment.

As regards the MDR; we found the MDR of the UPEC was 39.4%. This result is similar to other previous studies that indicated an increasing resistance to three or more classes of antibiotics, especially in developing countries (Sanchez et al., 2014).

As regards the Ceftriaxone resistance; Ceftriaxone resistance was shown to be the most common in the current study (62.25 percent). This can be explained by the recent emergence of ß-lactam resistance in nosocomial Enterobacteriaceae, which has become a severe problem worldwide, notably the increasing resistance to third-generation cephalosporins (Ceftriaxone) (Pfeifer et al., 2010). Ceftriaxone is therefore a less common first-line medication for UTIs, but it is essential for treating more serious infections (Abernethy et al., 2017).

Different resistance rates have been recorded in different geographical courtiers in studies of ceftriaxone resistance status. Pakistan (90 %) and Ethiopia (73 %) showed higher ceftriaxone resistance (Gashe et al., 2018; Kathia et al., 2020). Mexico (27.3%) and Jordan (55.1%), on the other hand, have lower resistance patterns for *E. coli* isolates against ceftriaxone (Ramírez-Castillo et al., 2018; Shakhatreh et al., 2019). Others, have identified lesser resistant *E. coli* isolates (20%) in Saudi Arabia (Aabed et al., 2021), (7.8%) in northern Brazil from 2007 to 2010 (Cunha et al., 2016), and 6.7 percent in 2013 in Zawiya, Libya (Abujnah et al., 2015; Cunha et al., 2016).

As regards TMP-SMZ**;** TMP-SMZ resistance was the second most common in this study (54.8%). It is used in practice a lot, and (Jancel & Dudas, 2002) say it should be the first choice for treating a simple UTI (cystitis).

Susceptibility patterns vary across geographical regions and alter over time, as previously mentioned (Prakash & Saxena, 2013). For example, approximately equal frequencies of UPEC isolates resistant to TMP-SXT were found (2018) (Alqasim et al., 2018; Cunha et al., 2016; Raeispour & Ranjbar, 2018). While Mongolia had more TMP-SMZ resistance in 2017, Mexico had 72.7 percent in 2018, while Jordan had 73.1 percent in 2019 (Munkhdelger et al., 2017; Ramírez-Castillo et al., 2018; Shakhatreh et al., 2019). Certain European countries reported a lower frequency of between 14.6 and 60 percent in 2019 (Kot, 2019); Switzerland reported 24.5 percent from 2012 to 2015 (Erb et al., 2018); 37.1 percent in France in 2016 (Lavigne et al., 2016). In different Libyan cities, *E. coli* isolates showed varying rates of resistance to TMP-SMZ throughout time, for example, 1994-1995/Tripoli (45%), 1996/Benghazi (81%), 2002- 005/ Sirte (36%), 2006-2008/ Benghazi (31%) (Ghenghesh et al., 2003).

According to (Abujnah et al., 2015), resistance to TMP-SMZ was 24.6 percent in 2013. Due to increased resistance to TMP-SMZ that has recently been documented in numerous countries, TMP-SMZ should not be used in empiric UTI treatment and the

maximum resistance that can be tolerated is 20% (Bartoletti et al., 2016).

As regards Nitrofurantoin resistance; Because nitrofurantoin's activity against commonplace causes of UTIs, such as *E. coli*, is well-documented, so in this study the resistance was found to be (18.26 percent). Nitrofurantoin resistance is uncommon in principle, and many MDR species remain vulnerable (Sanchez et al., 2014) These findings support the European Association of Urology (EAU) (Grabe et al., 2015) and International Clinical Practice Guidelines' recommendations that nitrofurantoin be used first-line for the treatment of uncomplicated UTIs (Grabe et al., 2015; Rowe & Juthani-Mehta, 2013).

This result was similar in low rate of resistance to nitrofurantoin as in Mexico (2013–2017) by (Ramírez-Castillo et al., 2018), in India was (12.7%). by (Prasada *et al*., 2019) and in Saudi Arabia found that the prevalence of nonsusceptible *E. coli* to nitrofurantoin was 15% (Alqasim et al., 2018). While in Tripoli, Libya 2003 was (25%) (Ghenghesh et al., 2003).

As regards* fluoroquinolones *resistance; The fluoroquinolones resistance rates in this study (ciprofloxacin 26% and levofloxacin 15.4%) suggest that fluoroquinolones antibiotics, such as ciprofloxacin and levofloxacin, are recommended for empirical oral antimicrobial treatment in uncomplicated UTIs (pyelonephritis) and are widely used in clinical practice against UTI pathogens such as *E. coli* (Bonkat et al., 2018; Drago et al., 2001), whereas various countries reported significant levels of fluoroquinolone-resistant *E. coli* (Drago et al., 2001). It is thought that the widespread use of fluoroquinolones in outpatients is the reason for the persistent increase in resistance to this medication. Thus, the use of ciprofloxacin as empirical therapy for UTI should be avoided, and the application of policy that restricts ciprofloxacin use should be enhanced, particularly in developing countries (Fasugba et al., 2015; Karam et al., 2019).

In agreement with our results, what was found in previous studies, which showed that fluoroquinolone resistance rates are always less than 20%, with a few cases of much higher resistance rates of 49% to 72% (Walker et al., 2016). For example, nearly similar results were found in Benghazi, Libya between 2006 and 2008; 17% were resistant to ciprofloxacin (Buzayan et al., 2010; Ghenghesh et al., 2003); in Switzerland, 17.4% were resistant to ciprofloxacin (Erb et al., 2018); in Brazil, 18.8% were resistant to ciprofloxacin (da Silva et al., 2017); in Zawiya city between 2012 and 2013; 23.1% were resistant to ciprofloxacin; and 19.2% to levofloxacin (Abujnah et al., 2015). In developed countries, the rates were much lower (5.1% in the USA, 10.5% in Germany, and 24.8% in France) than in developing countries (64.6% in Nepal, 58.1% in Mongolia, and 55.5% in Jordan) (Khatri et al., 2017; Munkhdelger et al., 2017; Shakhatreh et al., 2019).

While higher frequencies were reported in Saudi Arabia. 40% was resistance to ciprofloxacin (Alqasim et al., 2018). In Mexico, 47.3% was resistance to ciprofloxacin and 43.6 to levofloxacin (Ramírez-Castillo et al., 2018); in Pakistan, 60% was resistance to ciprofloxacin and 61.4% to levofloxacin (Ali et al., 2016).

As regards Amoxicillin-Clavulanic acid resistance; In our study, the percentage of *E. coli* resistance to Amoxicillin-Clavulanic acid was 47.11%. For this reason, amoxicillin-clavulanic acid is not indicated for empirical treatment due to the high prevalence of bacterial resistance (Bartoletti et al., 2016). Therefore, its treatment should be based on the susceptibility results of UPEC (Kot, 2019).The variance level of *E. coli* isolates resistant to amoxicillin-clavulanic acid among patient groups or geographical regions is unknown. For example, it was

reported higher in Jordan, where 2019 was 83.2% (Shakhatreh et al., 2019), in Pakistan, 2016 was 71% (Ali et al., 2016), and in Saudi Arabia it was 55% (Abernethy et al., 2017; Alqasim et al., 2018; Kot, 2019; Lavigne et al., 2016; Ramírez-Castillo et al., 2018) reported low resistance in France (36.6%), England (30%), Mexico (23.6%), and Poland (13.9%).

Strength: To best of our knowledge, this is the first study in Libya that study this topic during the COVID-19 pandemic, to cover large period of time more than 18 months (January 2021 to April 2022) in order to evaluate the rate of *E. coli* resistance to the chosen antimicrobial agents.

Limitations: Being a retrospective study and record based study may affect the quality of the collected data, and we cannot calculate the incidence and cannot prove. As a single city study, may limit the generalizability of the results. in other Libyan cities.

CONCLUSION

In Libya's Al-Bayda, during the COVID-19 pandemic, the following antibiotics had the highest antimicrobial resistance patterns: ceftriaxone, TMP-SMZ, amoxicillin + clavulanic acid, and ciprofloxacin, in that order. While levofloxacin and nitrofurantoin were the antibiotics with the highest sensitivity, age is a significant determinant of antimicrobial sensitivity patterns in E. coli urine isolates, while sex is only a significant determinant of antimicrobial sensitivity patterns in the TMP-SMZ, ceftriaxone, and nitrofurantoin treatments. Antibiotic resistance in UPEC is a severe concern in Libya that requires immediate attention from health officials.

RECOMMENDATIONS

1. The results of the current study demonstrates clearly that the problem of antibiotic resistance in *E. coli* treatment in Libya is a very serious problem that needs urgent attention by the health authorities.
2.For setting up a basis for clinical treatment of *E. coli* infections, readily available data (Antibiogram profiling) on antibiotic resistance patterns from annual reports of clinical laboratories should be used for the choice of appropriate antimicrobial therapy in patients with suspected UTI.
3.Alternatives to the commonly used antimicrobial for the treatment of UTI in Libya should be considered, particularly those with high resistance (ceftriaxone, TMP-SMZ and Amoxicillin + Clavulanic acid)**.** Thus, selection of appropriate antibiotics for the UTIs should start after establishing monitoring systems based on antibiotic susceptibility pattern of the causative isolate obtained (Karam *et al*., 2019; Sifaw Ghenghesh, 2003).
4.Further multicenter, and prospective research should be conducted either on the *E. coli* or other types of bacteria on a regular basis.

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Phylogenetic Analysis of Libyan Thyme (Thymus Capitatus) Inferred from The Morphological Traits

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Abstract: The genetic diversity of wild thyme (*Thymus capitatus*) which growing in southern parts of Al-Jabal Al-Akhdar region, Libya was studied by using cluster analysis of morphological traits (flower measurements). This study was aimed to establish the phylogenetic relationships based on floral parameters among accessions of thyme (*T.capitatus*). The five populations (accessions) of Libyan thyme were assigned into two clusters (clades) at the critical distance value of 22%. The 1st cluster contained three populations that were included white-flowered, dotted white-flowered and violet-flowered accession, then the 1st cluster was divided into two sub-clusters by the critical distance value of 5%, the first sub-cluster contained two populations (white-flowered, dotted white-flowered accession). While, the second sub-cluster contained one population (violet-flowered accession). The 2nd cluster contained two populations which were purple-flowered and mosaic-flowered accessions. In conclusion, The flower measurements can be a preliminary tool to classify Libyan thyme (*T.capitatus*), and floral parameters can be used in the classification of Libyan thyme accessions (populations).

التحليل الوراثي للزعتر الليبي (*Thymus capitatus*) المستنتج من الصفات المورفولوجية

الكلمات المفتاحية :
الزعتر؛
الزعتر البري؛
الصفات المورفولوجية؛
قياسات الزهرة؛
التحليل العنقودي؛
التنوع الجيني.

المستخلص : تم دراسة التباين الوراثي لنبات الزعتر البري(*Thymus capitatus*) ، والذي ينمو بالأجزاء الجنوبية لمنطقة الجبل الأخضر، في ليبيا باستخدام التحليل العنقودي لصفات المورفولوجية (قياسات الزهرة). تهدف هذه الدراسة إلى تحديد العلاقات التطورية القائمة على الصفات الزهرية لنبات الزعتر (*T.capitatus*).عدد خمسة عشائر طبيعية لنبات الزعتر الليبي حددت في عنقودين (فرعين) عند قيمة مسافة تباعديه 22% . العنقود الأول شمل ثلاثة عشائر: عشيرة النباتات بيضاء الأزهار وعشيرة النباتات بيضاء الأزهار المنقطة وعشيرة النباتات بنفسجية الأزهار. العنقود الثاني احتوي على عشيرتين: عشيرة النباتات أرجوانية الأزهار وعشيرة النباتات مزركشة الأزهار. علاوة على ذلك، فقد انقسم العنقود الأول إلى تحت عنقودين: الأول شمل عشيرتين هما عشيرة النباتات بيضاء الأزهار وعشيرة النباتات بيضاء الأزهار المنقطة، والثاني احتوي على عشيرة واحدة وهي عشيرة النباتات بنفسجية الأزهار. في الختام ، يمكن أن تكون قياسات الزهور أداة أولية لتصنيف الزعتر الليبي (*T.capitatus*) ، ويمكن استخدام الصفات الزهرية في نبات الزعتر للتعريف والتفريق بينهم.

INTRODUCTION

Since the time of Darwin, systematists have attempted to reconstruct phylogenies by looking at the distributions of character states in organisms. In general they have assumed that those organisms with more character states in common are more closely related and that overall similarity should reflect genealogy (Baker et al., 1998; Coombs et al., 1981; Duncan et al., 1980; Hennig, 1966). A major goal in systematic biology is to examine the relationships among a group of closely related organisms to determine their evolutionary history (Manos et al., 2001). A phylogenetic tree, also called an evolutionary tree, is a

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graph showing the evolutionary interrelationships among various entities that are believed to have a common ancestor. In this graph (a form of a cladogram), each node with descendants represents the most recent common ancestor of the descendants, and edge lengths correspond to time estimates (Ali and Mustafa, 2020). Traditional phylogenetic methods rely on morphological data obtained by measuring and quantifying the phenotypic properties of representative organisms (Lewis, 2001).

Many biologists agree that a phylogenetic tree of relationships should be the central underpinning of research in many areas of biology. Comparisons within or among plant species in a phylogenetic context can provide the most meaningful insights into biology. This important realization is now apparent to researchers in diverse fields, including ecology, molecular biology, physiology and evolution (Crane, 1985; Daly et al., 2001; Doyle & Donoghue, 1986; Doyle et al., 2008; Hall et al., 2002; Leht, 2009; Soltis & Soltis, 2000). One obvious importance is the value of placing organism in the appropriate phylogenetic context to obtain a better understanding of both patterns and processes of evolution. The most popular and frequently used methods of phylogenetics tree building is the unweighted pair group method with arithmetic mean (UPGMA). This method is simple, fast and has been extensively used in many studies to investigate genetic distance among populations or accessions determined by morphological traits (Doyle, 2013; Lewis, 2001 ; Ali and Mustafa; 2020).

Phenotypic characters have been and continue to be used as essential components in the final classification of living organisms. Till today, morphological characters have been the main descriptive tool characterize a given collection or germplasm, to identify and differentiate wild type populations (Ali et al., 2019; Daly et al., 2001; Doyle, 2013; Doyle & Endress, 2000; Doyle & Luckow, 2003; Ennouri et al., 2017; Schneider et al., 2009; Soltis & Soltis, 2003). Phylogenetic analyses based on phenotypic characters have been used to identify phylogenetic relationships in various plant species, including ferns (Kenrick & Crane, 1997), morning glory (Manos et al., 2001), peonies (Zhi-Qin & Pankai-Yu, 2003), corallinoidean taxa (Kim et al., 2007). Also, the genetic distance as well as genetic variation among accessions can be determined by using cluster analysis based on phenotypic traits (Hillis & Wiens, 2000; Wiens, 2004).

Thyme(*T.capitatus***)** is a perennial woody plant, 15 cm tall, with abundant stoloniferous branches. The inflorescences are composed of whorls of small, zygomorphic flowers. The pollination in *T.capitatus*isentomophilous, as in most *T.* species. The main pollinators are *Apismellifera* and some species of Bombus. *T.capitatus* is gynodioecious, as for most species of *T.*(Ali et al., 2019; Gruenwald et al., 2004). Many reports have shown that *T.capitatus* possesses biological properties: antimicrobial activity (Alves et al., 2000; Bounatirou et al., 2007; Ebrahimi et al., 2008), antifungal activity (Grayer & Harborne, 1994; Kalemba & Kunicka, 2003; Ricci et al., 2005) and antioxidant activity (Bounatirou et al., 2007; Ricci et al., 2005 ; Al-mustafa and Al-thunibat, 2008). Moreover, the honey from *T.capitatus* flowers is largely appreciated for its delicacy (Figueiredo et al., 2008).

Thymus capitatus is an endemic wild plant in Al-Jabal Al-khdar area, Libya (SWECO, 1986). *T. capitatus* growing in southern parts of Al-Jabal Al-khdar showed flower color polymorphism which results in five different phenotypes: white- flowered, dotted white-flowered, purple-flowered, violet-flowered and mosaic-flowered accessions (Ali et al., 2019).

The main objective of this study was to establish the phylogenetic relationships based on floral parameters among accessions of thyme (*T.capitatus*) which growing in southern parts

of Al-Jabal Al-khdar region, Libya.

MATERIALS AND METHODS

T.capitatus growing in south parts of Al-Jabal Al-khdar region has shown a stable flower-color polymorphism (Fig. 1). There are five patterns (five populations or accessions): white-flowered, dotted white-flowered, purple-flowered, violet-flowered and mosaic-flowered plants (Ali et al., 2019).In this investigation, 5 accessions (populations) of *T.capitatus* representing the diversity of thyme, were collected during flowering period (from mid June to the end of July) from different sites in Al-Jabal Al-khdar region, Libya . For each population, 10 individual plants were selected randomly. For each plant, five floral measurements were made on 5 healthy flowers. The total length of flower, stamen length, style length, calyx length were measured using mm scale. The ratio between the calyx length and the total length of flower is determined as the percent value .

Means and standard deviations were calculated according to standard methods (Manly, 1986 ; Ali and Mustafa; 2020). The data matrix was converted into a matrix of dissimilarity values using standard methods (Coombs et al., 1981; Lewis, 2001; Wiens, 2004). Unweighted pair-group method using an arithmetic average (UPGMA) cluster analysis was performed. The standardized mean values of every floral trait were used to perform the cluster analysis using appropriate procedures of the program SPSS (version 14). During tree construction process, all measurements of flower for five populations were unordered and equally weighted, with multi-states interpreted as uncertainty.

RESULTS

Name accessions (populations) and means of floral traits (flower measurements) of Libyan thyme (*T.capitatus*) were presented in Table 1. Flower measurements were flower length (ranged between 6.38 and 3.31 mm), Calyx length (ranged between 2.40 and 1.08 mm), Stamen length (ranged between 4.65 and 3.41mm), Style length (ranged between 5.37 and 4.20 mm) and Calyx/ flower length (ranged between 0.58 and 0.19 %).

Based on average values of floral traits (flower measurements), the five populations of Libyan thyme (*T.capitatus*) were classified into two clusters (clades) at the critical distance value of 22%, the 1st cluster (the early or medium-flowering cluster) and the 2nd cluster (the late flowering cluster). The 1st cluster (the early or medium-flowering cluster) contained three populations that were: white-flowered, dotted white-flowered and violet-flowered accession. Furthermore, the 1st cluster was divided into two sub-clusters by the critical distance value of 5%, the first sub-cluster contained two populations (white-flowered, dotted white-flowered accession) while the second sub-cluster contained one population (violet-flowered accession). On the other hand, the 2nd cluster (the late flowering cluster) contained two populations which were purple-flowered and mosaic-flowered accessions (Table 1 and Fig. 2).

Results of the present study showed that, there were a closer relationship between white-flowered and dotted white-flowered population than between either of them and any other populations of *T.capitatus*. These two populations first formed their own monophyletic group, and then together formed a monophyletic clade with violet-flowered population. In addition, purple-flowered and mosaic-flowered formed a monophyletic group, which was then joined by other thyme's populations.

In this study, the accessions that have large flowers size, were grouped into early or medium-blooming cluster, while the accessions that have small flowers size were clustered into late or medium- blooming cluster, based on floral traits cluster analysis.

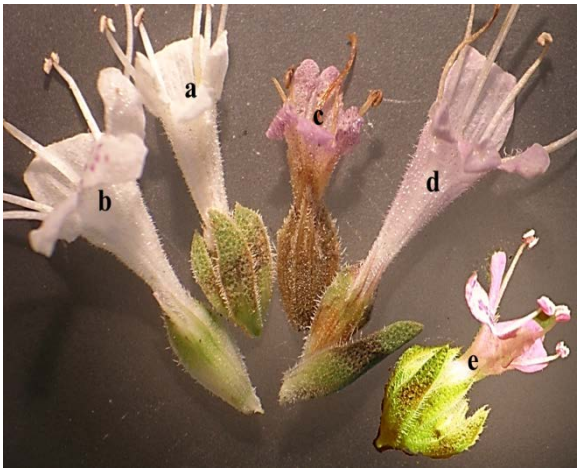

Figure: (1). Flower color polymorphism in Libyan thyme (*T.capitatus*):
(a) white- flower (b) dotted-white flower (c) purple flower (d) violet-flower and (e) mosaic-flower.

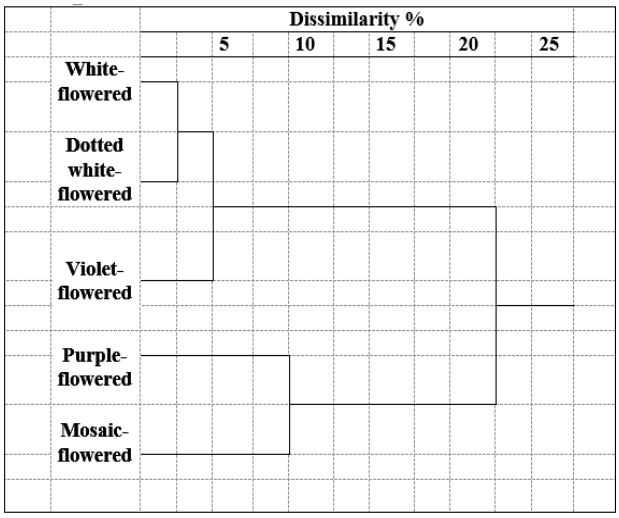

Figure: (2). Dendrogram for five accessions of Libyan thyme (*T.capitatus*) by floral traits data.

Table:(1). Means of floral traits (flower measurements) of the five accessions Libyan thyme (*T.capitatus*)

Accessions (Populations)	Blooming time	Means of floral measurements				
		Flower length (mm)	Calyx length (mm)	Stamen length (mm)	Style length (mm)	Calyx / flower length (%)
White-flowered	Early	5.83	1.08	3.41	4.46	.19
Dotted white-flowered	Early	5.65	1.26	3.55	4.20	0.22
Purple-flowered	Late	3.31	2.13	4.33	4.86	0.64
Violet-flowered	Medium	6.38	1.54	4.56	5.37	0.24
Mosaic-flowered	Late	4.15	2.40	4.65	4.53	0.58

Table:(2). Distance matrix of the Libyan thyme (*T.capitatus*) populations.

	Populations				
	White	Dotted white	Violet	Purple	Mosaic
White	0.00	0.33	1.30	23.45	18.31
Dotted white	0.33	0.00	1.67	18.93	14.79
Violet	1.30	1.67	0.00	25.35	17.25
Purple	23.45	18.93	25.35	0.00	2.51
Mosaic	18.31	14.79	17.25	2.51	0.00

DISCUSSION

The current study indicated that flower measurements (morphological characters) can generate a well-supported phylogenetic tree for Libyan thyme (*T.capitatus*). These results are in agreement with several studies, which demonstrated that morphological characters are informative for phylogenetic studies (Kenrick & Crane, 1997; Renzaglia et al., 2007; Schneider et al., 2002 ; Ali and Mustafa, 2020). It is common for morphological data to be considered less important than DNA sequence data in phylogenetic studies (Bowe et al., 2000; Endress & Igersheim, 2000). However, morphological characters (such as flower measurements) differ substantially from DNA sequence characters in their complexity and their frequency of evolutionary change. Many morphological characters including floral parameters show a much lower mutational rate

than DNA sequence and thus may be less prone to problems such as saturation (Cracraft & Donoghue, 2004; Donoghue & Yang, 2016; Endress & Doyle, 2009; Hillis & Wiens, 2000). Therefore, some phenotypic parameters are likely to be ideal phylogenetic characters because they allow us to identify single evolutionary events (Bateman et al., 2006; Lee & Palci, 2015; Mathews, 2009).

Moreover the results showed that phylogenetic analysis using floral parameters is an easy, inexpensive, useful and powerful approach in taxonomic purposes. Several previous studies demonstrated that phenotypic parameters were reasonable tool to build phylogenetic tree for many plant species (Doyle et al., 2008; Giribet, 2015; Hall et al., 2002; Lewis, 2001 ; Ali and Mustafa, 2020).

In this study, all of the large flower size accessions (populations) were grouped into early or medium-blooming cluster, while the small flower size accessions were grouped into late blooming cluster, based on flower measurement cluster analysis. In addition, the violet-flowered accession was assigned into unique sub- cluster. It can be suggested that floral parameter analysis is a useful tool in studying the difference in ecological type. Therefore, morphological trait analysis (such as flower measurements) may be helpful to identify patterns of combinations between the diverse ecological type variety groups (Ali et al., 2019; Doyle, 2013; Schneider et al., 2009).

The current results indicate that flower measurements (morphological characters) can be a preliminary tool to classify Libyan thyme (*T.capitatus*).Moreover, the present research has revealed that floral parameters can be used in the classification of Libyan thyme accessions (populations). This result was confirmed by other researches which reported that the morphological characters have an important role in the classification and identification for many species (Doyle, 2013; Manos et al., 2001; Zhi-Qin & Pankai-Yu, 2003 ; Ali and Mustafa, 2020).

The result of our cladistic analysis indicates that The success of a phylogenetic analysis depends upon the discovery of character sets that provide accurate information. This result was confirmed by other researches which reported that many character sets are molecular but the value of morphological data to reconstruct phylogenetic relationships was reasonable tool to build phylogenetic tree for many plant species (Davis et al., 1998; Doyle, 2013; Ennouri et al., 2017; Hu et al., 2018; Wiens, 2004; Wodniok et al., 2011 ; Bremer *et. al.*, 2009).

Phylogenetic tree based on morphological characters was also reported in other plant species like apple (Forte et al., 2002), Theaceae (Luna & Ochoterena, 2004), vicia (Leht, 2009) and olive (Ali and Mustafa, 2020). However, the phylogenetic tree based on morphological data could not reveal the genetic relationship among cultivars adequately (Keating et al., 2020; Lewis, 2001; Soreng et al., 2017). Furthermore, the morphological variation does not always reflect real genetic variation because of genotype-environment interaction and the largely unknown genetic control of polygenic morphological and agronomic traits (Ali et al., 2019; Ayed et al., 2015; Doyle, 2013; Wiens, 2004).

CONCLUSION

By using cluster analysis of morphological traits (flower measurements) for five natural populations *Thymus capitatus* (accessions) were assign into two clusters. The 1stcluster contained three populations that were white-flowered, dotted white-flowered and violet-flowered accession. Furthermore, the 1st cluster was divided into two sub-clusters distance value the first sub-cluster contained two populations (white-flowered, dotted white-flowered accession) while the second

sub-cluster contained one population (violet-flowered accession). On the other hand, the 2nd cluster contained two populations which were purple-flowered and mosaic-flowered accessions

Duality of interest: The authors declare that they have no duality of interest associated with this manuscript.

Author contributions:, Ali Contributed data, Performed the analysis; Ali and Mustafa Drafting the manuscript; Mustafa Conceived & design of the study, Collected the data; Ali, Mustafa and Omar Performed the analysis; Both Mustafa and Omar approval of the final version of the manuscript.

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Prevalence and Distribution of Pine Processionary Moth (*Thaumetopoea pityocampa*) in Shahat's Aleppo Pine (*Pinus halepensis*) Plantations, Al-JabalAl-Akhdar, Libya

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Abstract: The current study aims at investigating the spatial distribution of pine processionary moth (PPM) in parts of Al-Jabal Al-Akhdar region in Libya (in Shahat area). Two *Pinus halepensis* stands in the area (in Al-Mansurra and the ancient city of Cyrene) were chosen for field data collection. Growth parameters for pine trees including; tree height, crown height, diameter at breast height, stand density level, location within the stand, health condition, in addition to the observed number of PPM nests per tree were recorded. Pearson correlation coefficient analysis and variance analysis were applied to assess the relationship between obtained variables, and evaluate growth conditions for both stands. Results revealed relatively better growth conditions at Al-Mansurra site compared to Cyrene site. Moreover, PPM nests occurrence was positively correlated with trees growing isolated or on the stand edge (r = 0.54, P value <.001), and negatively correlated with tree height (r = - 0.4, P value <.001) emphasizing more dispersal rate among young pine trees. Crown height, dbh, and tree's health condition showed no significant effect on PPM spread in the area. In-depth investigations of the population dynamics of PPM are highly recommended to provide insights into their spatial distribution in the region.

انتشار فراشة جادوب الصنوبر (*Thaumetopoea pityocampa*) وتوزيعها في مشجرات الصنوبر الحلبي (*Pinus halepensis*) في منطقة شحّات، الجبل الأخضر، ليبيا

الكلمات المفتاحية:
فراشة جادوب الصنوبر، *Thaumetopoea pityocampa*، التوزيع المكاني، الصنوبر الحلبي، منطقة شحات، الجبل الأخضر، ليبيا.

المستخلص: تستهدف الدراسة الحالية معرفة التوزيع المكاني لفراشة جادوب الصنوبر (PPM) في أجزاء من منطقة الجبل الأخضر في ليبيا (تحديدًا في منطقة شحات). تم إختيار مشجَّرين للصنوبر الحلبي في المنطقة (قرية المنصورة وموقع المدينة الأثرية في شحات) لإجراء الدراسات الحقلية. خصائص النمو لأشجار الصنوبر بما فيها: ارتفاع الأشجار، وارتفاع التاج، والقطر عند مستوى الصدر، وكثافة المشجر، وموقع الأشجار ضمن المشجر، والحالة الصحية، فضلًا عن عدد أعشاش آفة PPM لكل شجرة تم تسجيلها. استُخدم معامل ارتباط بيارسون وتحليل التباين لتقييم العلاقة بين البيانات المعطاة، ولتقييم ظروف النمو لكلا المشجرين. النتائج أوضحت– إلى حدٍّ ما– وجود ظروف نمو أفضل لمشجر المنصورة مقارنة بظروف النمو الموجودة بمشجر الموقع الأثري بشحات. كما أوضحت النتائج أن وجود أعشاش آفة PPM وانتشارها ارتبط بشكل إيجابي مع الأشجار النامية بصورة معزولة أو موجودة على حافة المشجر (r = 0.54 P value <.001) وبشكل سلبي مع ارتفاع الأشجار (r = - 0.4, P value <.001) وهذا يدل على وجود معدلات انتشار أعلى بين الأشجار الصغيرة في العمر. لم تسجّل بيانات ارتفاع التاج، والقطر عند مستوى الصدر، والحالة الصحية للأشجار أي تأثير معنوي على انتشار آفة PPM في منطقة الدراسة. يُنصح بشدة بإجراء دراسات مفصلة عن ديناميكية وسلوك مجتمعات آفة جادوب الصنوبر؛ لمعرفة المزيد عن سلوكها وتوزيعها المكاني في المنطقة.

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INTRODUCTION

The pine processionary moth (PPM) *Thaumetopoea pityocampa* (Denis & Schiffermüller)(Lepidoptera, Thaumetopoeidae) is one of the most harmful pests to conifer species in the world (Kesdek et al., 2020; Otsu et al., 2018; Sevim et al., 2010), and one of the main pine defoliators in the Mediterranean region (Camarero et al., 2022; Devkota & Schmidt, 1990; Régolini et al., 2014; Sangüesa-Barreda et al., 2014). PPM distribution is usually controlled by the significant drop in temperature during winter seasons (Battisti et al., 2005). However, evidences were documented for the expansion of PPM in the upper northward of the Mediterranean as a consequence of climate change (Battisti et al., 2005; Robinet et al., 2007; Rosenzweig et al., 2007). This pest causes severe damage throughout the autumn, but especially in the winter when larvae feed on pine needles (Castagneyrol et al., 2014). By that time, they can be easily distinguished on pine trees with conspicuous white silky nests on the upper and lower branches that face the sunlight. They feed mostly during nighttime and hide in their nests during daytime away from their natural predators (Huchon & Demolin, 1970). Even when the percentage of defoliation is relatively low, PPM outbreak might decrease the annual growth rate of infected trees in the short term and interrupt the growth of newly emerging saplings (Hódar et al., 2003; Jacquet et al., 2013; Jacquet et al., 2012). The severe defoliation caused by PPM as well as other abiotic agents may have a negative impact on the economic value of pine stands (Sangüesa-Barreda et al., 2014). Furthermore, it may increase the susceptibility to other pine pests such as bark and wood boring beetles (Masutti & Battisti, 1990; Zamoum, 2002). In spring, caterpillars make a head-to-tail procession in which they march down from the crown of trees to bury themselves into selected sunny exposed soils for pupation. They remain buried until the moth's emergence in the next summer or for a longer time in what is known as "semi-voltine cycle" (Martin et al., 2021) where they stay for prolonged pupal diapause for one or more years. The emergence of an adult moth occurs in summer, and the life cycle continues after reproduction when the short-lived female moth searches for a prospective host tree for oviposition (Castagneyrol et al., 2014). Besides the severe defoliation effect, the urticating hairs "setae" which PPM caterpillars release as a defensive mechanism might lead to serious allergic reactions in humans and animals such as dermatitis and anaphylactic reactions (Moneo et al., 2015; Vega et al., 2003).

As a Mediterranean basin country, the distribution of pine processionary moth in Libya was briefly described in literature (El Mokhefi et al., 2016; Kerdelhué et al., 2009; N Avtzis et al., 2016) with limited sufficient details regarding their distributional range and current outbreak status. (Kerdelhué et al., 2009) classified the PPM found in Cyrenaica into one of the sub-clades derived from the ENA clade. The existing similarities between Greek and Libyan mitochondrial DNA haplotypes might indicate the original source of PPM in Greece according to (N Avtzis et al., 2016).

The scarcity of information about the origin, distribution, and impact of PPM on pine trees, in addition to the raised public-health awareness due to the approximation of pine plantations to urban areas, were among the drivers of this work. Therefore, the objectives of the current study are set to evaluate the distributional range and patterns of PPM infestation on pine trees in Shahat area in Al-Jabal Al-Akhdar region. Moreover, we examine whether tree height, size, and location have an impact on the degree of severity and spread of PPM in pine trees in the subjected area of study.

MATERIALS AND METHODS

The Study Site Description: This study was carried out in two pine plantations in Shahat

municipality, Al-Jabal Al-Akhdar region in eastern Libya (Cyrenaica). The first site is a pine plantation in Al-Mansurra town (N= 320. 83' 74'', E= 210. 84' 14''), (Altitude = 405m). The site was established in 1958 surrounding Shahat Hospital of Chest and Respiratory Diseases. It is classified as a pure pine plantation (more than 95% of the basal area is *P. halepensis*) and covers an area of approximately 13 ha. The second site is a mixed conifer and broadleaf plantation that was established in the early 1940s during the Italian colonization era in the ancient city of Cyrene (N= 320 49' 12'', E= 210 51' 48''), (Altitude = 620m). *P. halepensis* compresses the majority of the stocking area (around 75% of the total number of species) followed by *Cupressus empervirens*, *Eucalyptus camaldulensis*, and other evergreen species. The forested area around the ancient city of Cyrene is estimated to be between 70- 80 ha and was established mainly to protect the archaeological ruins and heritage sites in the city. Both study sites are located in one of the most humid and cold places in Al-Jabal Al-Akhdar region with an average annual precipitation of 600 mm and an average temperature of 20 C^0 (OMU report, 2005). The predominant soil types in the study areas are the red Mediterranean soils of Ferrisiallitic Red (Rhodoxeralfs) and shallow calcareous Rendzinas (Rendolls Lithic) soils (Mahmoud, 1995).

Sampling and methodology: Field work for this study took place in April 2022 at the later instar of larvae phase, right before the procession started. At each of the study sites, five quadrate sampling plots (15x15m) were established and measurements were recorded for each pine tree included in the study design. The parameters measured were as follows; tree height (using Silva Clinometer at a fixed distance from the tree trunk) (Avalos et al., 2005; Wright et al., 1997), crown height (the total foliar part of the sampled tree using also Silva Clinometer), Diameter at breast height (dbh) (using diameter tape at 1.3m), presence of PPM nests (visual observation), number of total PPM nests per sampled tree, and notes of the current health status of the sampled tree and its location (inside, on the edge, or away from the stand centre). Additionally, 3 core samples were extracted from three different pine trees within each sampling plot using Pressler's increment borer to estimate the average age of each site following standard dendrochronological procedures (Stokes & Smiley, 1968). Among the 10 plots sampled, two plots (one on each plantation) were established in young *P. halepensis* stands (less than 10 years old) to estimate the infestation percentage among young pine cohorts. The young pine stand in Al-Mansurra plantation was self-regenerated in a protected open area on the site, while the ancient city of Cyrene site was established in a reforestation project by the City Coordination Association (NGO) back in 2013. Data collected in this project were grouped and extrapolated to give a better representation of the whole study area. Descriptive statistics and graphs were generated to illustrate the current stand outstanding.

Statistical Analysis: Variance analysis (ANOVA) was performed and Pearson correlation coefficients were calculated for nest occurrences and stand variables of height, crown height, dbh, tree location, and health condition to investigate the relationship between PPM distribution and these growth parameters. All statistical analyses were performed using IBM SPSS Statistics software 28.0.

RESULTS

Current stands condition: As illustrated in (Table 1, Fig.1), Al-Mansurra pine plantation expands on an area of 13 ha and is classified as a pure *P. halepensis* stand (more than 95% of basal area). The average number of trees/ha is estimated at around 506.6 tree/ha. Mean tree height is 13.2 m, mean crown height is 6.5m, and mean diameter at breast height is 38.1 cm. In general, the site is in good condition (arguably better than Cyrene site) in terms of density, tree height, size, vigour, and health condition. Emerging seedlings of pines are observed, more concentrated in low-dense and open areas since the site is protected against invaders and

livestock. PPM is considered the only pest affecting pine trees on the site with no evidence of other disturbing biotic agents.

In the ancient city of Cyrene site, *P. halepensis* is the predominant species with 75% of the basal area and expands on an area of around 75 ha. The density of the plantation is relatively low compared to Al-Mansurra site with an average number of trees/ha of 311, mean tree height is 12.6 m, mean crown height of 5.6 m, and mean diameter at breast height of 33 cm. Several biotic and abiotic factors have a deteriorative effect on the growth of pine trees on the site. For instance, the Mediterranean pine engraver beetle (*Orthotomicus erosus* Wollaston) is responsible for the death of many pine trees, and combined with PPM outbreak and other abiotic agents are causing severe damage resulting in the increasing number of fallen and dead logs on the site. Both stands of Al-Mansurra and Cyrene are classified as unmanaged stands, where there are no signs of silvicultural treatments or any sort of management approaches (thinning, clearcutting, pruning, etc.) applied since the establishment date.

Table (1): Description of current pine status in both sites in the study area. Younger pine cohorts (less than 10 years old) in both stands were excluded from the given results

Plantation name	Coordinates	Altitude(m)	Percentage of *P. halepensis* in the stand	No of trees/ha	Stand age	Average PPM nest occurrence per tree	Density level	Stand health condition
Al-Mansurra	N= 32. 83 E= 21. 84	400-412	95%	506.6	62 ± 3	3.22	High dense stand	Good
Cyrene	N= 32. 49 E= 21. 51	560-625	75%	373.3	49 ± 19	3.25	Moderate dense stand	low

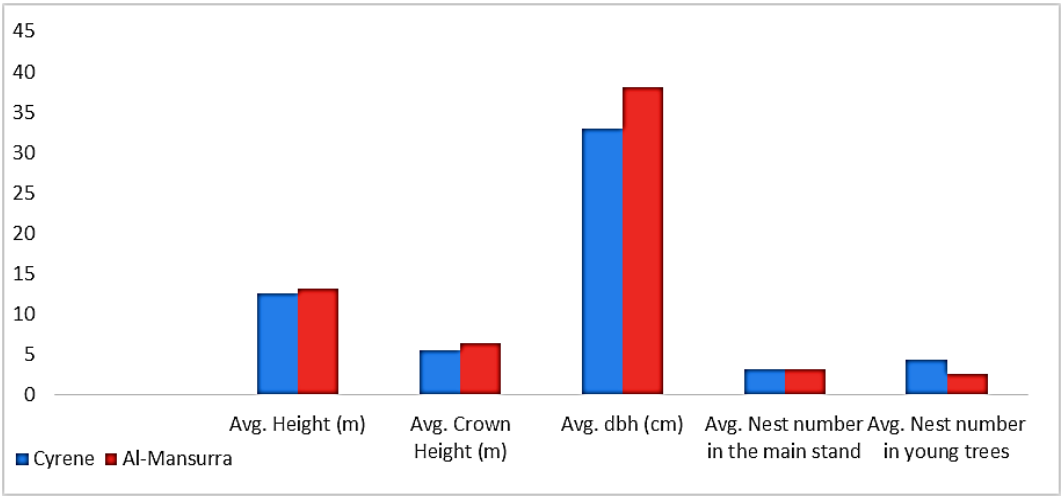

Figure (1): Stand characteristics in both study sites of Cyrene and Al-Mansurra

***T. pityocampa* distribution:** PPM silky white nests were observed on a large scale in both plantations (Fig. 3). Descriptive analysis of the obtained data showed similar distributional patterns in both sites in the number of nests per pine tree (3.25 and 3.22 for Cyrene and Al-Mansurra sites, respectively). For younger pine cohorts, the dispersal of PPM in Cyrene was significantly higher than that of Al-Mansurra site (4.42 and 2.61 nests per tree, respectively). The infection rate of spread differs greatly between both sites on stand level. In Al-Mansurra

site, around 38% of pine trees have at least one observed PPM nest while in Cyrene site, on the other hand, more than three-quarters of pine trees (75%) are affected by PPM outbreak. Moreover, younger pine trees in both sites exhibited more PPM nests spread compared to older cohorts, with infection percentages of 100% and 85% for both Al-Mansurra and Cyrene sites, respectively. Linear regression and bivariate correlation were applied to investigate possible relationship between the percentage of nest existence and stand parameters such as tree height, crown height, diameter at breast height, tree location within the stand, and vigour condition. The results (Table. 2) suggest a significant positive correlation between the presence of PPM nests and the tree location within the stand (r = 0.54, P value <.001). Pine trees on the stand edge or in areas where they are exposed to full sunlight are more vulnerable to PPM attack, while inside-stand trees have a better chance of surviving PPM spread. On the contrary, a significant negative correlation was found between PPM nest's presence and the height of trees (r = - 0.4, P value <.001), which indicates a high infestation rate among young and small-size trees compared to dominant and co-dominant trees in both stands. No significant correlations were found among the effect of crown height, diameter at breast height (dbh), or vigour condition on nest occurrence in pine trees as shown in (Table.2, Fig. 2).

Table:(2): Descriptive statistics of some of the trees and stand parameters in correlation with PPM occurrence in the study area

Trees parameters in relation to PPM nest occurrence	Mean	Std. Deviation	Pearson Correlation	Sig. (2tailed)
Tree height (m)	10.2	4.064	-0.4	<.001
Crown height (m)	4.14	2.762	-0.1	.323
Diameter at breast height (cm)	24.42	14.885	-0.18	.069
Tree location	1.616	0.488	0.54	<.001
Vigour condition	3.05	0.896	-0.03	.731

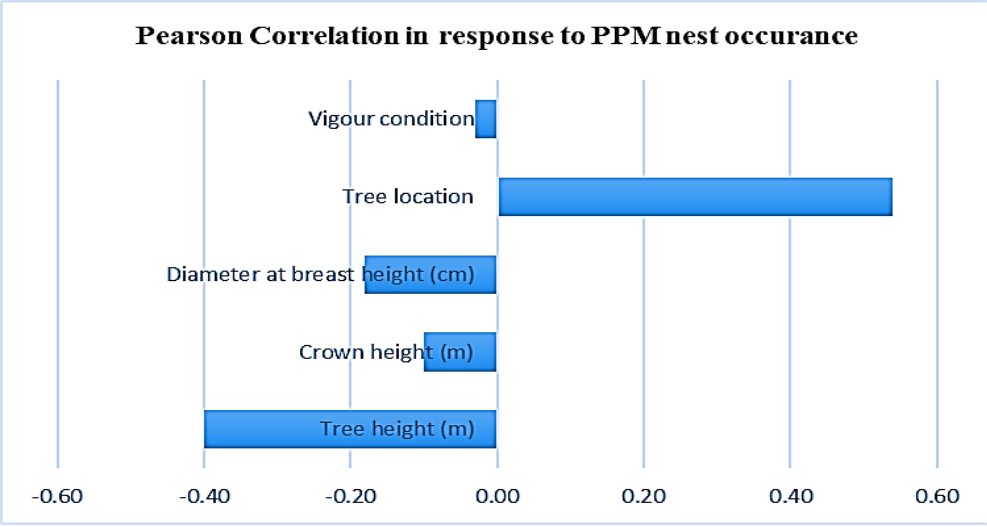

Figure (2): Correlation significance between PPM nest occurrence and some of the studied stand variables.

Figure (3): (A) shows *T. pityocampa* spread among young P.halepensis trees. **(B)** shows *T. pityocampa* nest a few hours before the procession starts. Both images were taken in Cyrene site, Shahat, Libya.

DISCUSSION

Despite the limited information available regarding the origin and distribution of PPM in Libya, this pest has a history of negatively affecting the growth of pine trees (mainly *P. halepensis*) in many forested areas in the country, especially in Al-Jabal Al-Akhdar region, where the species is the most favored in reforestation projects. The expansion of PPM toward higher latitudes and altitudes in areas that previously were unaffected by the pest (Stastny et al., 2006) as a consequence of climate change (Battisti et al., 2005; Benigni & Battisti, 1999; Goussard et al., 1999; Robinet et al., 2007; Rosenzweig et al., 2007) has led to more comprehensive studies investigating population distributional patterns and infestation mechanisms of the insect. On the contrary, different distributional patterns and surviving rates were observed in the southern edge of PPM distribution (in North Africa). Climate warming may contribute to increasing mortality rates of the pest's early life stages in Tunisia (Bourougaaoui et al., 2021). Moreover, PPM nests have disappeared from areas in the far southern-edge range of Tunisia where it was previously proven to exist. One of the observed impacts that climate change has on PPM populations is the change in the usual timing at which larval procession starts (Bonsignore et al., 2015). For instance, field data for this study took place in April right before the start of the larval procession. Usually, this process happens much earlier in late February or early March in the region. However, the rainy season of this year (2022) lasted longer than usual and led to a delay in the procession time of this pest.

PPM outbreak tends to be higher near the forest edge or in isolated trees (Démolin, 1969). The ideal preference for this best is selecting taller and edge-located trees for colonization (Démolin, 1969). The findings of this study were in parallel with PPM preference, where the highest and only positive nest occurrence correlation found was with *P.halepensis* trees growing on the stand edge ($r = 0.54$). Higher solar radiation and warmer temperatures might be responsible for such observed spatial

distributions (Buffo et al., 2007). The density level of pine trees in Al-Mansurra site (506.7 tree/ha) was relatively higher than that of Cyrene site (373.3 tree/ha). This might explain the higher infestation rate, especially in younger pine cohorts, in Cyrene Plantation. These results were in agreement with (Geri et al., 1985) who found that PPM tent density declined with increasing density levels in *P. nigra* and *P. sylvestris*, and (Régolini et al., 2014) who reported similar results with *P. pinaster* growing in parts of France. However, (Lombardero et al., 2012) found no significant impact of thinning on PPM density in young *P. pinaster* stands in Spain. The distribution of PPM was notably higher in younger pine trees in both study sites. The percentage of PPM nest occurrence was negatively correlated with tree height (r =- 0.4). Observations of PPM nests are common among young pine trees in the study area as well as in nearby places since the species is extensively used in reforestation programs, shelter belts, and road-sides plantations. These findings were in contrast with (Régolini et al., 2014) who reported a significant increase in PPM attack with increasing tree height, and (Dulaurent et al., 2012) who stated that PPM infestation was not common among young *P. pinaster* stands. A long-term outbreak of PPM may cause a decrease in both height and size of affected trees compared to healthy unaffected pine trees (Carus, 2004; Jacquet et al., 2012).

The expansion of PPM in both natural and artificial pine plantations, more specifically during the last decade in the study area and the whole region of Al-Jabal Al-Akhdar, has raised concerns among researchers regarding the potentially harmful effect of this pest on pine populations. Its vast dispersal among young trees might be the trigger for more cautious approaches to prevent its expansion. However, scientific works regarding the subject in the region have not been published so far, and this study might highlight the importance of more investigation to be carried on. Proper management approaches might be a key factor in maintaining stand density at beneficial levels to help decrease PPM infestation. The outcome of this work can be applied to all pine plantations in Al-Jabal Al-Akhdar region since they are homogeneous and share the same growth and climatic conditions. *P.halepensis* is an important tree species in the region (OMU report, 2005; Alsanousi & Ali, 2018), and its ability to survive drought and the semi-arid harsh environments (Quézel, 2000) makes it the most favourable species in afforestation projects (Schiller, 2000). Indeed, the species compresses more than 60% of Libya's total plantation basal area. Maintaining pine stands in the country at sustainable and historical levels is essential for future conservation approaches.

CONCLUSION

T. pityocampa populations were found in many of the natural and artificial *P.halepensis* stands in Al-Jabal Al-Akhdar region, in eastern Libya. This pest has a long history of affecting pine trees in the study area, although no scientific works have documented its dispersal and impact on these pine stands. Ecologists, foresters, and local references declared that PPM populations had increased rapidly especially during the last decade. Despite that the severe defoliation damage of this moth has not been observed or documented on a large scale in the region, cautious and protective measures should be applied since the majority of pine stands in the area are under stress either by biotic or abiotic agents. This study found a high prevalence of PPM nests among isolated and stand-edge pine trees compared to inner stand trees. Most of pine trees in the region are in pure, moderate to low, dense stands which make them more susceptible to PPM infestation. Interestingly, the most alarming finding in this study is the spread of PPM nest among young pine trees even those within high-dense stands. This may negatively affect the growth of these young pine cohorts and threaten their survival capability to replace old stands. Practical management approaches to sustain and protect these young stands are needed now more

than ever in order to achieve long-term sustainability of *P.halepensis* stands in the region.

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Sensitivity of Some Apple Varieties Grown in Regions of Al-Jabal Al-Akhdar to Apple Scab Disease

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Apple Scab disease; *Venturia inaequalis* ; Al Jabal Al-Akhdar; Libya.

Abstract: The study aimed to know the extent of infection of apple varieties grown in Al-Jabal Al-Akhdar regions with apple scab disease during the seasons 2018-2019. The results showed the incidence of the disease was higher in August in Al-Bayda, Shahat, and Al-Marj, at a rate of 73, 72, and 61.7%, respectively, while it was higher in September in the regions of Al-Kuf and Qandula, at a rate of 79.6, and 73.2%, respectively. The study showed the highest infection rate was among the local variety, with a rate of 86% and a severity of 41%, followed by Starking variety, with 77% in 2018 season. As for 2019 season, the Local variety was the most infected, with a rate of 80% and a severity at 43%. The study showed the relationship between the percentage of the disease and its severity with the environmental conditions, results showed that the relationship between the incidence of disease and temperature in Al-Bayda was weak (r = 0.15), as well as between the severity of the disease and relative humidity (r = 0.081). We conclude that the cultivars in the study area are sensitive to the disease, and Golden Delicious cultivar is more susceptible to infection.

حساسية عدة أصناف التفاح المزروعة في بعض مواقع الجبل الأخضر للإصابة بمرض جرب التفاح

الكلمات المفتاحية :
جرب التفاح؛ *venturia inaequalis*؛ الجبل الأخضر؛ ليبيا.

المستخلص : استهدفت الدراسة معرفة مدى إصابة أصناف التفاح المزروعة بمناطق الجبل الأخضر بمرض جرب التفاح خلال الموسم 2018–2019 م، وأوضحت النتائج أن أعلى نسبة للإصابة بالمرض سُجِّلت في شهر أغسطس بمناطق البيضاء وشحات والمرج بمعدل 73، 72، 61.7 % على التوالي، في حين جاءت أعلى في سبتمبر في مناطق الكوف وقندولة بمعدل 79.6،73.2% على التوالي، وفي دراسة حساسية الأصناف المزروعة بالمنطقة أوضحت النتائج أن أعلى إصابة كانت في الصنف البلدي بمعدل 86٪ وبشدة إصابة 41٪، يليه صنف ستاركنغ Starking بنسبة 77% في موسم 2018، أما في موسم 2019 فكان الصنف البلدي أكثرها إصابة بمعدل 80٪ وبشدة إصابة 43 ٪، بدراسة العلاقة بين نسبة المرض وشدته مع الظروف البيئية أظهرت النتائج أن العلاقة بين نسبة المرض ودرجة الحرارة في منطقة البيضاء كانت علاقة ضعيفة (r= 0.15) كذلك بين شدة المرض والرطوبة النسبية (r= 0.081) . نستنتج أن الأصناف المزروعة بمنطقة الدراسة حساسة للإصابة بمرض جرب التفاح، والصنف Golden delicious هو الأكثر عرضة للإصابة.

المقدمة

تعد شجرة التفاح (*Malus domestica* L.) التابعة للعائلة التفاحية من الأشجار الخشبية متساقطة الأوراق (Brown, 1992)، ويضم جنس الـ *Malus* من 20 إلى 30 نوعًا وفقًا لما ذكره (Geibel et al., 1999). ثمار هذا النبات مفضلة بوصفها غذاءً؛ لغناها بالمواد الكربوهيدراتية والبروتين والمواد الدهنية والبكتينية، فضلًا عن الأحماض العضوية والأملاح المعدنية، كما يمكن استخدامها في صناعات أخرى مثل العصائر والخل (Korban & Tartarini, 2009; Vejl et al., 2003)، يمتاز نبات التفاح بتنوع أصنافه المزروعة في المناطق المعتدلة من العالم (Harris et al., 2002)، أما في ليبيا فقد أُدخل فيها العديد من الأصناف منذُ حوالي 30 عامًا بمنطقة الجبل الأخضر وفقًا لأمانة الزراعة في مشروعها الزراعي بمناطق زراعة التفاح، ويُذكر أن عدد المزارع المنتجة

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للتفاح بالمنطقة- وفق كش وفات وزارة الزراع ة- تق در ب . . . 697 مزرع ة، وبع دد أش جار مثم رة تق در ب . حوالي 921 ألف شجرة (Suleiman, 2014)، ووفقًا لمنظمة الأغذية والزراعة الدولية قُدِّر الإنتاج ب . . 31 ألف طن في عام 1995، وكان إنتاج التفاح في الفترة من ع ام 2000-2019 يُقدر بحوالي (300) ألف طن، ويتضح من البيانات الصادرة عن منظمة الفاو أن مساحة زراعة التفاح انخفضت من 850 هكتارًا سنة 1985 إلى 353 هكتارًا في عام 2015. إن أكثر الأصناف زراع ة بمن اطق الجب ل الأخضر هي صنف Delicious Golden بعدد 57.4 ألف شجرة، إي ما يعادل 40 % من إجمالي الأصناف؛ وذل ك لما يمتاز به هذا الصنف من كثرة إنتاج، وكونه ملقحًا جيدًا، يليه الصنف Stark Delicious بعدد 43.3 ألف شجرة ما نسبته 30%، والصنف Jonathan بعدد 23.7 ألف شجرة، يليه الصنفان Ein Shamir Anna بم ا يع ادل 13% (ELmsalaty, 2013). ينتشر مرض جرب التف اح ف ي العديد من مناطق زراعة التفاح، فقد سُجِّل انتشار الم رض في الكثير من دول الع الم مث ل مص ر (Radwan & Hassan, 2019) والمغ رب (Lahlali et al., 2019) وفي أوروبا وآسيا Xu وآخرون (2008)، وفي الولاي ات المتحدة Biggs وآخرون، (2010). وهناك العدي د م ن أصناف التفاح المقاومة لمرض ج رب التف اح، ومنه ا: Crimson gold و Crimso topaz (Crassweller، 2018). فضلًا عن أصناف التفاح الحاوية على جين rvi6 مثل صنف Liberty و Florina (Papp et al. 2020). ويتسبب مرض جرب التف اح ع ن الفط ر *Venturia inaequalis* (Wilcox, 1993)، حيث صُنف هذا الجنس بواسطة (COOKE، 1880)، ويمتاز بأن ل ه ح التين: إحداهما: الترممي ة (Cke) *V. inaequalis* والأخ رى: الحالة التطفيلية ف ي حال ة الط ور الن اقص (Wallr) *Fusicladium dendriticum* (Menon, 1956). وتسعى هذه الدراسة إلى تقييم حساسية أصناف من أش جار التفاح المزروعة بمنطقة الجبل الأخضر لم رض ج رب التفاح.

مواد وطرق البحث

حصر الم رض: حُدِّدت زيارة ميدانية ابتداءً من شهر يوليو حتى نهاية الموسم في أواخر شهر سبتمبر، لعدد ستة مواقع مُتباينة الارتفاع عن سطح البحر، وحُدِّدت مس احة 100م² لكل موقع، باستخدام جهاز GPS مع تحديد خطوط الطول ودوائر العرض والارتفاع عن سطح البحر (جدول 1)

الجدول (1). خطوط الطول والعرض والارتفاع عند مس توى س طح البحر لمواقع الدراسة بمنطقة الجبل الأخضر

المواقع	خط الطول	دائرة العرض	الارتفاع (م)
شحات	21.879560	32.787911	641
البيضاء	21.772006	32.777463	612
مسة	21.622250	32.749615	505
وادي الكوف	21.576192	32.716744	460
قندولة	21.579885	32.541507	622
المرج	20.831534	32.502895	330

الظروف المناخية : استخدمت البيانات المناخية الص ادرة من الموقع https://power.larc.nasa.gov/ خلال الفترة الممتدة من مايو إلى أكتوبر لعامي 2018 و 2019م ، كما هو موضح في الجدول (2).

دراسة حساسية أص ناف التف اح المزروع ة بالمنطق ة للمرض: لحساب درجة المرض بموقعي (البيضاء وشحات) خلال شهر سبتمبر من موسمي (2018، 2019)، قُ دِّرت نسبة وشدة الإصابة للأصناف المزروعة ذات عمر ف وق 30 سنة وه ي:Golden delicious ، Red delicious ، starking، Ein shamir، فض لًا ع ن الص نف المحلي Local varaity، حيث قُسِّم الحقل إل ى مربع ات بمساحة 100م^2، وجُمعت 50 ورقة من كل شجرة، حُسبت نسبة الإصابة وفقً ا لمعادل ة (James, 1971) = (نس بة الإصابة= عدد الأوراق المص ابة/العدد الكُلِّ ي ل لأوراق 100X)، أما شدة الإصابة فقُدِّرت وفقًا لمقي اس الم رض الذي يشتمل على درجات الإصابة (شكل 1) وفقًا للمعادل ة الت ي اقترحه ا(Horsfall & Heuberger, 1942) =

مجموع (عدد النباتات في كل درجة من درجات مقياس المرض X رقم الدرجة)/ (عدد نباتات العينة كلها X أعلى درجة إصابة) X 100.

الجدول (2). درجات الحرارة والرطوبة النسبية في مواقع الدراسة من 2018-2019

الشهر	المرج			
	رطوبة نسبية (%)		درجة حرارة (م°)	
	2018	2019	2018	2019
5	12.8 ± 63.8	7.5±71.1	2.7±21.7	2.0±18.8
6	10.0± 67.8	8.6±70.5	6.3±30.9	2.5±23.9
7	5.7±71.1	5.1±72.4	0.8±25.7	1.1±25.8
8	5.4±70.2	3.9±70.6	0.8±26.9	0.5±26.6
9	3.9±72.1	5.7±69.8	1.1±25.1	1.2±25.2
10	7.2±71.1	5.7±68.4	1.3±22.3	1.3±23.5
	البيضاء والمناطق المجاورة			
5	13.8±55.2	7.6±65.5	3.0±21.8	2.5±18.6
6	11.8±59.9	9.6±62.1	2.0±24.1	2.6±23.8
7	7.0±64.4	5.6±64.9	1.0±25.2	1.4±25.3
8	6.7±65.4	3.7±65.3	1.0±26.1	0.8±25.8
9	3.8±71.1	6.4±67.0	1.4±23.6	1.4±24.0
10	6.7±71.3	7.1±66.9	1.5±20.7	1.6±22.1

درجة حرارة (م°) والرطوبة % المقاسة في مواقع الدراسة خلال السنوات 2018-2019

* ±الخطأ القياسي http:// Power.larc.nasa.gov

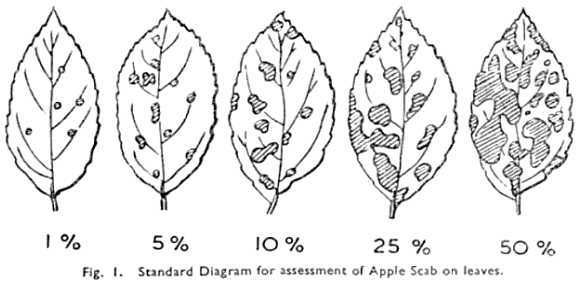


Fig. 1. Standard Diagram for assessment of Apple Scab on leaves.

الشكل (1). المقياس المستخدم في الدراسة وفقًا لـ Croxall وآخرين، 1952)

علاقة الحرارة والرطوبة بدرجة المرض: لدراسة هذه العلاقة جُمعت عينات من أوراق الأشجار لصنف Red delicious في الموقعين البيضاء والمرج خلال شهر أغسطس من الموسم 2018، حيث جُمعت 50 ورقة من كل شجرة لخمس أشجار من كل مزرعة بالموقعين، وحُسبت نسبة الإصابة وشدتها، كما ذُكر سابقًا، كما تم تحديد العلاقة بين تطور الأعراض المرضية والظروف البيئية السائدة من حرارة ورطوبة بكلا الموقعين عن طريق حساب معامل الارتباط للعلاقات المختلفة كما في دراسة لـ Tomerlin & Jones, 1982).

النتائج والمناقشة

بيَّنت نتائج حصر كمية المرض على أشجار التفاح في مواقع الدراسة الموضحة في الجدول (3) أن شهر أغسطس سجَّل أعلى معدلات نسبة إصابة لكل من شحات والبيضاء ومسة والمرج، في حين سُجِّلت أعلى نسبة في شهر سبتمبر في المواقع الكوف وقندولة بمعدلات 79.6% و 73.2% على التوالي. وفيما يتعلق بشدة الإصابة كانت أعلى نسبة في شهر أغسطس لأغلب المواقع عدا شحات التي سجَّلت النسبة الأعلى في سبتمبر بمعدل 31%، ويُعزى تفاوت نسبة الإصابة بالمرض وشدتها من شهر إلى آخر لعدة عوامل، منها الظروف البيئية من حرارة ورطوبة والتي كانت في أعلى معدلاتها في شهر أغسطس، وذلك يتوافق مع الدراسة التي أجراها (Menon, 1956) والتي بيَّن فيها أن نشاط الفطر المُمرض يتأثر بالظروف الجوية مثل الحرارة فيزداد تجرثمه في فصل الصيف عند درجات حرارة 25 م°، وكذلك تتفق مع دورة حياة الفطر التي أظهرت أن الفطر ينشط كطور كونيدي في فصل الصيف (Wilcox, 1993) ويتفق كذلك مع دراسة Singh، وآخرين (2015) أوضحوا أن الجرب قد بدأ في التطور بسرعة كبيرة في منتصف شهر أغسطس، وأن الجرب كان شديدًا على الصنف Golden Delicious ومتوسط على الصنف Red Delicious.

عند دراسة حساسية أصناف التفاح المختلفة للإصابة بالجرب، يتضح من الجدول (4) نسبة الإصابة وشدتها على الأصناف في المناطق المدروسة (البيضاء، شحات)، ومن الجدول يظهر بأن نسبة الإصابة وشدتها على الصنف

المحلي كانت أعلى من الأصناف الأخرى في موسمي 2019، 2018، يليه صنف Starking بنسبة إصابة بلغت 77% وبشدة إصابة بلغت 39% في الموسم 2018، أما في الصنف Red delicious فبلغت نسبة الإصابة 77% في الموسم 2019 وكانت شدة الإصابة للصنف Golden delicious 34% في موسم 2019، وأعلى درجات إصابة على الصنف المحلي في منطقة الدراسة؛ لأن هذا الصنف من الأصناف المهملة التي لا يقوم المزارعون بكثرة الإجراءات الوقائية عليها.

الجدول (3). نسبة وشدة الإصابة بمرض جرب التفاح في مواقع الدراسة.

المواقع	7		8		9	
	نسبة الإصابة	شدة الإصابة	نسبة الإصابة	شدة الإصابة	نسبة الإصابة	شدة الإصابة
شحات	67.8	26.4	72.0	30.8	65.0	31.0
البيضاء	52.1	21.0	73.7	35.3	65.3	27.1
مسة	49.1	23.3	71.9	34.2	62.9	19.8
وادي الكوف	56.8	22.0	70.6	33.6	79.6	24.3
قندولة	58.9	26.3	64.1	27.0	73.2	22.6
المرج	52.4	19.0	61.7	24.6	55.4	20.2

أقل فرق معنوي عند5 %= المواقع =12.6، الأشهر 27.5 ، الأشهر * المواقع 25.1

أُجريت دراسة مشابهة في المغرب من قبل (Lahlali et al., 2019) تم فيها تقدير نسبة ظهور المرض وشدته على أصناف التفاح التجارية بمقاطعة سايس، وأظهرت الدراسة أن شدة المرض كانت أعلى ظهورًا بالصنف Golden delicious بنسبة 40.31% يليه صنف Starking بنسبة 20.45 %. ويظهر بالشكل (2) أعراض الإصابة بالمرض على أصناف التفاح بمنطقة الجبل الأخضر التي أُجريت عليها هذه الدراسة على شكل تبقعات سوداء اللون وفلّينية الملمس وتشوهات بالمظهر الخارجي، تتفق هذه الأعراض مع الأعراض التي ذُكرت في عدد من الدراسات (Doolotkeldieva & Bobusheva, 2017; Khajuria et al., 2014؛ Ziems 2019). وقد ذكرت دراسة لـ (Spinelli et al., 2010) أن مثبطات ثنائي أوكسجينيز

كان لها دور كبير في تخفيض الإصابة بمرض جرب التفاح على صنف Golden Delicious وذلك في تجارب حقلية والصوبات الزجاجية، ويعد المصدر الرئيس لمقاومة أصناف التفاح لمرض جرب التفاح هو الجين Rvi6 والذي يتم الحصول عليه من صنف النبات *Malus floribunda* (Sieb) وقد وُجد بأن هذا الجين وتعريفه على أنه مقاوم للفطر الممرض *Venturia inaequalis* في أوروبا (Papp et al., 2020).

الشكل (2) أعراض الإصابة بمرض جرب التفاح على ثمار التفاح المزروعة بمنطقة الجبل الأخضر

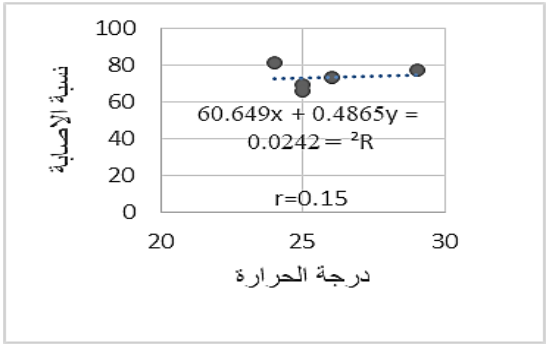
الجدول (4). نسبة وشدة الإصابة بمرض جرب التفاح على الأصناف المزروعة

الصنف	شحات			
	2018		2019	
	نسبة الإصابة	شدة الإصابة	نسبة الإصابة	شدة الإصابة
Red Delicious	68	29	77	31
Golden Delicious	71	26	67	34
Starking	77	39	64	28
Shamir	56	19	63	32
التفاح البلدي	86	41	80	43
	البيضاء			
Red Delicious	63.04	30.0	65.3	28.2
Golden Delicious	76.8	39.1	77.7	40
Starking	58.1	26.0	50.7	28.1
Shamir	70	32.7	78.9	37.8
التفاح البلدي	63	23	61	29

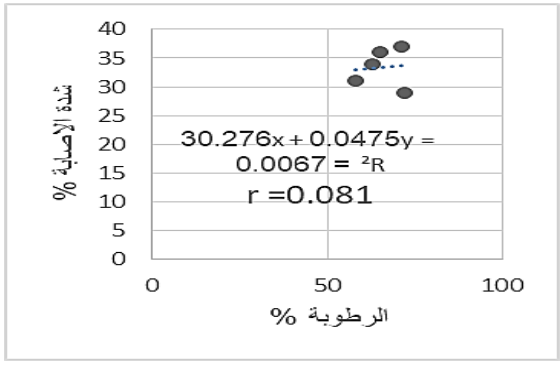
أقل فرق معنوي عند 5% LSD

نسبة المرض		شدة المرض	
الأصناف	11.31	الأصناف	8.44
السنة	13.7	السنة	10.26
الأصناف*السنة	13.05	الأصناف*السنة	7.48

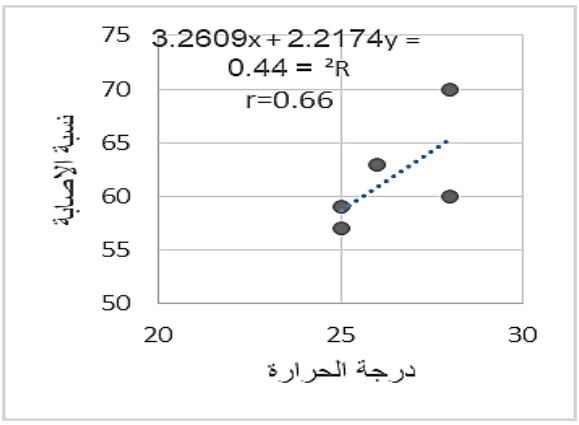
وبدراسة العلاقة بين شدة الإصابة والظروف المناخية المسجلة في موقعي البيضاء والمرج أثناء شهر أغسطس 2018 وحساب معامل الارتباط مع الظروف البيئية، يظهر بالشكل (3-أ) والذي يبين علاقة ضعيفة بين نسبة الإصابة ودرجة الحرارة بمنطقة البيضاء والتي كانت r=0.15، وفي الشكل (3-ب) العلاقة بين شدة المرض والرطوبة بمدينة البيضاء وكانت العلاقة بينهما ضعيفة كذلك r= 0.081 ، وفي الشكل (3-جـ . . .) تتضح العلاقة بين نسبة الإصابة ودرجات الحرارة بمنطقة المرج والتي كانت علاقة قوية، حيث كانت قيمة r=0.66، أما بالنسبة إلى شدة الإصابة والرطوبة (3- د). فكانت علاقة متوسطة؛ لأن الارتباط لم يتجاوز 0.6، وفي دراسة لعلاقة المرض مع الظروف البيئية قام بها (Tomerlin & Jones, 1982) أوضح أن تبقعات الجرب تطورت على النبات بعد مرور 3 أيام عند درجات حرارة بلغت 20 مئوية، وكذلك عند تعرضها للرطوبة العالية قبل ظهور الأعراض بأيام قليلة.



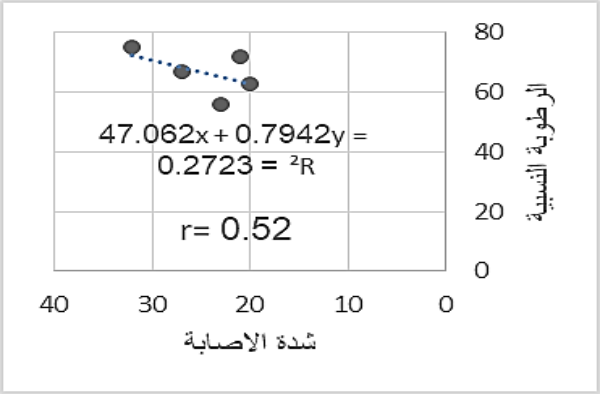
الشكل (3-أ). الارتباط بين نسبة الإصابة ودرجة الحرارة في منطقة البيضاء



الشكل (3-ب).الارتباط بين شدة الإصابة والرطوبة في منطقة البيضاء



الشكل (3-جـ .). الارتباط بين نسبة الإصابة ودرجة الحرارة في منطقة المرج



الشكل (3-د). الارتباط بين شدة الإصابة والرطوبة في منطقة المرج

أخلاقيات البحث :

هذا البحث جزء من رسالة ماجستير للباحث الاول وتحت اشراف الباحثين الثاني والثالث، كما ان البيانات والصور أصيلة وليست مقتبسة.

ازدواجية الاهتمام: يوجد تضارب في المصالح.

مساهمات المؤلف: متساوية بين المؤلفين.

التمويل: لا يوجد تمويل لدعم هذه المخطوطة.

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Effect of Seedling Date and Plant Density on Growth and Yield of Local Red Onion Variety *Allium cepa* L

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Keywords: Nutrition area; Local red onion variety; Planting date; Productivity indicators.

Abstract: The experiment was carried out in the General Commission for Agricultural Research, Syria. during the seasons 2019/2020 in order to investigate the effect of seedling date and plant density on the growth and yield of the local red onions variety and getting the bulbs directly without going through the stage of bulblets. Onion seeds were planted on 3 dates, with an interval of two weeks between dates (15/9, 30/9, 15/10). The seedlings were planted in the field on these dates (30/10, 15/11, 30/11) and with 3 plant densities (40, 20, 14 plant/m^2). The results indicated that the first date had a significant difference in indicators (height of plants, number of leaves, bulb weight, onion diameter, productivity (68.69 cm, 7.89 leaf/plant, 199.3 g, 7.87 cm, 2130 kg/m^2, respectively). As for the plant density, the plant density (14 plant/m2) had a significant difference to all studied indicators (69.15 cm, 7.66 leaf/plant, 238.1 g, 7.74 cm, 1667 kg/m^2, respectively). As for the interaction there was a significant difference of the first planting date with third plant density, with the possibility of adopting this shared treatment in shortening the life cycle of onions of the local red variety.

تأثير موعد الشتل والكثافة النباتية على النمو والإنتاجية للصنف الأحمر المحلي لنباتات البصل Allium cepa L.

الكلمات المفتاحية : المساحة الغذائية؛ صنف البصل المحلي الأحمر؛ موعد الزراعة؛ مؤشرات الإنتاجية.

المستخلص : نُفّذ البحث في الهيئة العامة للبحوث العلمية الزراعية– سورية خلال الموسم 2020/2019 بهدف دراسة تأثير موعد الشتل والكثافة النباتية في نمو وإنتاجية صنف البصل الأحمر المحلي، والحصول على الأبصال الأمهات مباشرة لاختصار مرحلة إنتاج البصيلات القزح. تمت زراعة بذور البصل في 3 مواعيد وبفاصل أسبوعين بين المواعيد (15 سبتمبر، 30 سبتمبر، 15 أكتوبر) وزُرع الشتل في الأرض الدائمة بمواعيد (30 أكتوبر، 15 نوفمبر، 30 نوفمبر) وبثلاثة كثافات نباتية (40، 20، 14 نبات/$م^2$). بينت النتائج تفوق الموعد الأول معنويًا في مؤشرات ارتفاع النبات، عدد الأوراق، وزن وقطر البصلة، الإنتاجية (68.69 سم، 7.89 ورقة/النبات، 199.3 غ، 7.87 سم، 2130 كغ/$م^2$ على التوالي)، أما الكثافة النباتية فقد تفوقت الكثافة (14 نبات/$م^2$) في جميع المؤشرات المدروسة سابقًا معنويًا (69.15 سم، 7.66 ورقة/النبات، 238.1 غ، 7.7.4 سم، 1667 كغ/$م^2$ على التوالي)، أما التفاعل فقد كان له تأثيرٌ معنوي، فقد تفوق موعد الزراعة الأول مع الكثافة النباتية الثالثة في المؤشرات المدروسة، مع إمكانية اعتماد هذه المعاملة المشتركة في اختصار دورة حياة البصل صنف الأحمر المحلي.

المقدمة

ينتمي البصل Allium cepa L. إلى الفصيلة الثومية Alliaceae (Chase et al,2009)، وعُرف البصل منذ ما يقارب من 4700 سنة، وتعد آسيا الوسطى (أفغانستان، كازخستان، طاجكستان، أوزبكستان) الموطن الأصلي له، وزرعه المصريون القدماء على امتداد نهر النيل منذ القدم، ومن ثم انتشرت زراعته في منطقة حوض البحر الأبيض المتوسط والذي يعد موطناً ثانوياً له (Vavilov,1956).

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تُعد الأبصال والأوراق الخضراء الجزء الاقتصادي في نبات البصل، واستخدمت منذ القدم لغايات غذائية وصحية في العديد من البلدان، وبلغت المساحة المزروعة من البصل في سورية (6089 هكتارًا) بمتوسط إنتاج (4504 كغ/هكتار) (الإحصائية الزراعية السنوية لوزارة الزراعة والإصلاح الزراعي في سورية، 2020).

تملك الأصناف المحلية في سورية بمخزونها الوراثي المتراكم عبر السنين أهمية كبيرة في عملية التحسين الوراثي، فهي تلقى قبول كلٍّ من المزارع والمستهلك، فضلًا عن كونها متأقلمة مع الظروف البيئية السائدة في مناطق زراعتها، ومقاومة للكثير من المسببات المرضية والحشرات المنتشرة في هذه المناطق (الهيأة العامة للبحوث العلمية الزراعية، 2015)، ويقع على عاتق العاملين في مجال التحسين الوراثي الحفاظ على هذه الأصناف، هذا ويتميز البصل المحلي كصنف البصل الأحمر المدروس بأنه ثلاثي الحول، ففي السنة الأولى تُزرع البذور لإنتاج بصيلات القزح (القنار أو البصيلات صغيرة الحجم)، والسنة الثانية تُزرع بصيلات القزح لإنتاج البصل العادي (الأمهات)، والسنة الثالثة تُزرع الأبصال الأمهات لإنتاج البذور، في حين يمكن زراعة البصل المستورد في عروتين ربيعية وخريفية ويمكن إنتاج الأبصال في موسم واحد، إلا أن القدرة التخزينية للبصل المستورد ليست بالجودة التي يتميز بها البصل المحلي (بطحوش، 2019)، ونظرًا إلى طول الفترة اللازمة للحصول على الأبصال الأمهات من البصل المحلي وما يترتب عليه من شغل الأرض لعدة سنوات، فضلًا عن التكاليف المرتفعة لمستلزمات الإنتاج هذا بدوره ساهم في عزوف كثير من المزارعين عن زراعة البصل المحلي وزراعة البصل المستورد، وهو ما يستدعي القيام بالعديد من الأبحاث التي من شأنها اختصار دورة حياة المحصول؛ بهدف المحافظة على البصل المحلي كمادة وراثية مهمة، ومنعها من الاندثار، وزيادة قدرته التنافسية مع البصل المستورد.

ويعد البصل من محاصيل الجو البارد، فقد توصل (Madisa,1994) إلى أن زراعة بذور البصل في منتصف شهر مارس يعطي الإنتاجية الأفضل 43 طن/هـ. مقارنة مع الزراعة المبكرة في فبراير (31 طن/هـ)، أما الزراعة المتأخرة في أبريل فقد أعطت إنتاجية (23.6 طن/هـ)، وبلغت نسبة الشمرخة (24 و7%) على التوالي ولم يلاحظ ظهور شماريخ في شهر أبريل، أما (Kretschmer, 1994) فقد بيّن أن 90% من بذور البصل تنبت تحت درجات حرارة (10– 25 °م)، وفي تشيلي درس (González, 1994) تأثير موعد الزراعة لثلاثة أصناف من البصل، حيث تمت الزراعة بأربعة مواعيد (7 يوليو، 21 يوليو، 5 أغسطس، 20 أغسطس، 4 سبتمبر) ولوحظ أن الإنتاجية الأعلى كانت عند موعد الزراعة بتاريخ 21 يوليو (52.42 طن/هكتار) والأقل عند الزراعة في الموعد 4 سبتمبر (0.84 طن/هـ)، كما وُجد في تجربة ثانية أن تأخير موعد الزراعة من يوليو إلى سبتمبر في تشيلي سبَّب زيادة ثخانة عنق البصلة، وعليه تزداد احتمالية الإصابة بأمراض ما بعد الحصاد وخلال فترة التخزين (Wright, 1997). وأوضح كلٌّ من(Cramer, 2003) أن الزراعة المبكرة لشتول البصل ينتج عنها زيادة معنوية في ارتفاع النبات وعدد الأوراق مقارنةً مع مواعيد الزراعة المتأخرة، وأشار (Ansari, 2007) أن بذور البصل المزروعة في يناير تنمو بعد /22/ يوم بمتوسط درجة حرارة 17.7 °م، أما البذور المزروعة في فبراير فتحتاج إلى /10/ أيام للإنبات بمتوسط حرارة (24.7 °م)، وتنمو بعد /7/ أيام عند الزراعة في مارس عند حرارة (34.7 °م).

في بنغلادش بيّن (Ud-Deen, 2008) عند زراعة بذور البصل بثلاثة مواعيد (10/30، 11/15، 11/30) بهدف دراسة معدل نمو الأبصال، أن موعد الزراعة المبكر (10/30) ساهم في إنتاج أبصال قطرها كبير (5.11 سم)، أما الزراعة في الموعد 11/30 فكان قطر الأبصال (4.50 سم)، أي أن الأبصال الأصغر حجمًا سجلت في الزراعة المتأخرة عند انخفاض الحرارة وقصر طول النهار.

وبدوره لاحظ (Ibrahim, 2010) انخفاضًا في الإنتاجي ة من الأبصال الجافة من 48 إلى 20 طن/ه . عند ت أخير الشتل من شهر أكتوبر إلى مارس.

بيّن (Abu-Rayyan et al., 2012) أن 70% من بذور البصل تنبت عند متوسط درجات الحرارة (7.5- 30°م)، ووجد (Patil et al., 2012) أن الإنتاجية الأعل ى م ن البصل بلغت (37.5 طن/ه كتار) عن د الش تل بموع د (11/15) بينما الإنتاجية الأقل (14.3 طن/ه .) عند الشتل بموعد (1/15). أوضح (Hamma, 2013) عند دراسة تأثير موعد زراعة بصيلات البصل على النمو والإنتاجية، أن موعد الزراعة بتاريخ 10/15 تفوق معنويًا في ارتفاع نباتات البص ل وع دد أوراقه ا (39.33 س م، 11.29 ورقة/نبات، على التوالي)، أما موع د الزراع ة بت اريخ 11/29 فكان الأقل في الصفات السابقة المذكورة (29.78 سم، 3.58 ورقة/نبات، على التوالي)، ولم يلاحظ وج ود فروق معنوية ب ين موع دي الزراع ة بت اريخ 10/15 و10/30 من حيث الإنتاجية من الأبصال الجافة (4.345، 4.287 كغ/ه كتار، على التوالي)، ف ي ح ين سُ جّلت الإنتاجية الأقل ف ي موع د الزراع ة 11/29 (3.670 كغ/ه . كتارًا).

ب .يّن (Gagopale & Gesine, 2013) أن مواعي .د الزراعة المبكرة لشتول البصل أعطت زيادة معنوية ف ي ارتفاع النبات وعدد الأوراق وحج م الأبص ال. أوض ح (Ashagrie et al., 2021) أن زراعة الأبصال في بداية أكتوبر ساهم في زيادة معنوية في إنتاج البذور في وح دة المساحة بنسبة 21.7 % مقارنةً مع الزراعة ف ي بداي ة نوفمبر.وجد (Misra et al., 2014) أن الزراعة المبكرة للبصل بتاريخ 11/25 أعطى أعلى نسبة من المادة الجافة للأبصال، وسجّلت الزراعة بموعد متأخر 1/10 أقل نسبة للمادة الجافة. وأشار (Mehri et al., 2015) إلى وج ود تأثير معنوي لموعد زراعة البصل على كلٍّ من مؤش ري ارتفاع النبات وعدد أوراقه عند الزراعة في شهر سبتمبر، مقارنةً مع النباتات المزروعة في شهر نوفمبر.

أكّد (Aboukhadrah et al., 2017) زي ادة محت وى أبصال البصل من المادة الجافة (17.07%) عند التبكي ر في موعد الزراعة بتاريخ 12/15 مقارنةً بموعد الزراعة المتأخرة 1/1 و1/15 (15.92%،13.89%) على التوالي، وكذلك زيادة نسبة المادة الجافة عن د الزراع ة بكثاف ة زراعية 30 نبات/م^2 (16.37%) مقارن ة بالكث افتين 45 نبات/ م^2 و60 نب ات /م^2 (15.85%، 14.65%، عل ى التوالي). أشار (Khan et al., 2020) عند زراعة شتول البصل بع دة مواعي د (15 ن وفمبر، 30 ن وفمبر، 15 ديسمبر، 30 ديسمبر، 14 يناير، 29 يناير) أنه كلما تأخر موعد الشتل في الأرض الدائمة انخفضت مؤشرات النم و والإنتاجية (قطر البصلة ووزنها)، وأعلى ارتفاع للنب ات وع .دد الأوراق والم .ادة الجاف .ة (54.51 س .م، 8.53 ورقة/نبات، 15.57 % على التوالي) كان عند الشتل ف ي موعد 15 نوفمبر، في حين بل غ وزن البص لة الأكب ر 26.26 غ عند الشتل في الموعد 15 ديسمبر.

في البرازيل ب يّن (Torres et al., 1986) أن زراع ة شتول البصل أعطى إنتاجية تراوحت ب ين 20.3 و35.8 طن/ هكتار مقارنةً ب . 10.3 طن/هكتار والناتجة من زرع البصل المباشر، تتوافق ه ذه النتيج ة م ع م ا وج ده (Motallebi et al., 2001) من زي ادة الإنتاجي ة م ن البصل عند الزراعة عن طريق الشتول مقارن ةً بالبذ ر المباشر في الأرض، وفي المكسيك أكّد كلٌّ من (Warid & Loaiza, 1993) ازدياد إنتاجي ة الأبص ال القابل ة للتسويق مقارنةً بطريقة زراعة البذور مباشرة في الأرض، كما أوضح (Pessala, 1990) أن طريقة زراعة ب ذور البصل مباشرة في الحقل أعطى أبصالًا ذات حجم أقل من الأبصال الناتجة عن زراعة الشتول. تمتلك الكثافة النباتية تأثيرًا كبيرًا على الإنتاجية البيولوجية والإنتاجية الاقتصادية للمحاصيل، إذ إن الكثافة تحدِّد م دى تع رُّض النبات ات للضوء واستفادتها منه في تكوين المادة الجافة، إن الكثافة المثلى لأي محصول ليست ثابتة بل تتغير تباعًا للظ روف البيئي .ة والأص .ناف، ففـ .ي المملك .ة المتح .دة درس

(Mettananda & Fordham, 1999) تـ أثير الكثاف ات الزراعية (10، 20، 30، 40 نبات/م^2) على عـ دد أوراق شتول البصـ ل، وتبـ يَّن أن عـ دد الأوراق فـ ي الشـ تول المزروعة على كثافة منخفضة (10، 20 نبات/م^2) تفوقت معنويًّا على مثيلاتها المزروعة عند الكثافة العاليـ ة (30، 40 نبـ ات/م^2) حيـ ث ازداد العـ دد مـ ن 3.7 إلـ ى 5.1 ورقة/شتلة.

درس كلٌّ من (Rumpel & Felczyński, 2000) تأثير الكثاف ـ .ات النباتيـ . .ـة (20، 40، 60، 80، 100، 140 نبات/م^2) على إنتاجية البصل الجاف، ووجدا أن الإنتاجية ازدادت من 20.5 طن/هـ كتار إلى 32.8 طن/هـ . كتار عند زيادة الكثافة من 20 إلى 80 نبات/م^2). وبيّن (Khan et al., 2002) أن زيادة الكثافة النباتية يقلل مـ ن طـ ول النبات وعدد الأوراق. أوضح (Jilani, 2004) عند دراسة تأثير الكثافة الزراعية (20، 30، 40 نبات/م^2) على عـ دد وطول أوراق /5/ أصناف من البصل، أن عـ دد وطـ ول الأوراق عند الكثافة 20 نبات/م^2 (12.05 ورقة، 37.99 سم، على التوالي) تفوّق معنويًّا عند الزراعة على الكثاف ـة النباتية 40 نبات/م^2 (9.99 ورقـ ة، 33.43 سـ م، علـ ى التوالي).

أوضح (Harris et al., 2016) عند زراعة البصل على مسافة 8*8 سم مع إضافة سماد الأزوتـ ي بمعـ دل 150 كغ/هـ . قد ساهم في زيادة معنوية في متوسط وزن البصلة مقارنةً مع الزراعة على المسافات المدروسـ ة الأخـ رى 10*10 سم و10*12 سم. وبيّن (أبو بكر، 2019) عنـ د دراسة تأثير المسافات الزراعية علـ ى نبـ ات الثـ وم أن الزراعة على مسافة 12.5 سم بين النباتات ساهم في زيادة معنوية في مؤشرات النمو الخضـ ري وقطـ ر البصـ لة، وانخفضت إنتاجية المحصول الكلي مقارنةً مع المسـ افات (5، 7.5، 10 سم). أوضح (Dorcas et al., 2012) أن زيادة الكثافة النباتية للبصل فـ ي وحـ دة المسـ احة مـ ن 100.000 نبات/هـ إلى 500.000 نبات/هـ يسبِّب انخفاضًـ ا في متوسط وزن البصلة من 58.22 غ إلى 40.04 غ على التوالي وانخفاض قطر البصلة من 4.56 سم إلـ ى 2.83 سـ م. فسـ ر (Naser et al., 2013) انخفـ اض العائـ د الاقتصادي في الكثافات المنخفضة على الرغم من زيـ ادة المؤشرات الأخرى المدروسة على نبات الفول، بأن كـ ل نبات على حدة يعطي الحدود القصوى من الإنتاج، ولكـ ن لن يكون هناك العدد الكافي من النباتات للوصول إلى العائد الاقتصادي الأمثل من وحدة المساحة واستغلال المـ وارد البيئية المتاحة بالشكل الأمثل.

وعليه يعد هذا البحث محاولة لدراسة تأثير موعد الشـ تل وكثافات الزراعة المختلفة في نمو وإنتاجية نباتات البصل صنف الأحمر المحلي وإمكانية إنتاج الأبصـ ال الأمهـ ات مباشرة دون المرور بمرحلة بصيلات القزح، مع المحافظة على الخصائص النوعية والإنتاجية.

الهدف من البحث:

– دراسة أثر موعد الشتل والكثافة النباتية في نمو النباتات لدى صنف البصل الأحمر المحلي والصفات الإنتاجية كمًّا ونوعًا.

– تحديد موعد الشتل والكثافة الأمثـ ل لزراعـ ة البصـ ل المحلي لإنتاج الأمهات من البصل خلال موسم واحد.

المواد وطرق البحث

– المادة النباتية: استُخدم صنف البصل الأحمـ ر المحلـ ي (مرحلة البذور)، والذي يتميز بأبصال بلبلية الشكل، لـ ون القشرة الخارجية أحمر بصلي، لون اللب أبـ يض، الطعـ م حرّيف، مصدر البذور من الهيأة العامة للبحـ وث العلميـ ة الزراعية.

– موقع التنفيذ: تم تنفيذ البحث في محطة بحوث الغوطـ ة التابعة لإدارة بحوث البستنة/الهيأة العامة للبحوث العلميـ ة الزراعية، دمشق– سورية خلال الموسـ مين الـ زراعيين 2020/2019، وتقع المحطة جنوب شرق دمشـ ق، تبعـ د عنها (15 كم)، على ارتفاع 610 متر عن سـ طح البحـ ر تتميز بشتاء بارد ورطب، وصيف حـ ار وجـ اف، تربـ ة

المحطة طينية ثقيلة، pH= 7.8 قاعدية، محتواها من المادة العضوية جيد، نسبة كربونات الكالسيوم عالية غير مالحة، محتواها من الفوسفور والبوتاس جيد.

المعاملات وطريقة العمل:

أ- زراعة البذور: قام الباحثون بزراعة البذور في صوانٍ فلينية بمقدار بذرتين في كل عين، خ لال المواعي د (15 سبتمبر، 30 سبتمبر، 15 أكتوبر)، وقُلعت الش تلات بع د حوالي 35-40 يومًا، أي بعد وصول الشتلات إلى الحجم المناسب (يك ون ع دد الأوراق 3-4 أوراق) (س لطان، 1983)، كما استُبعدت الش تلات الرفيع ة والمجروح ة والمصابة بالحشرات.

ب- الزراعة في الأرض الدائمة: جُهِّزت الأرض للزراعة بإجراء العمليات الزراعية كافة، من فلاحة وتخطيط، ث م زراعة الشتول في كل موعد من مواعيد الشتل المدروسة (30 أكتوبر، 15 نوفمبر، 30 نوفمبر) وذلك ضمن قط ع تجريبية مساحتها 1 م^2 تحوي 4 خطوط بفاصل 50 س م بين الخط والآخر، وب . 3 مكررات لكل معاملة، وتم الرَّيُّ بعد الزراعة مباشرةً، وبعد ذلك نُظّم الـ ري ك ل 7 أي ام حسب الظروف الجوية السائدة، وقُدِّمت للتجربة الخ دمات الزراعية كافة خلال موسم الزراعة، كما زُرعت الش تول بثلاث كثافات كما يأتي: (40 نبات/ م^2، 20 نبات/م^2، 14 نبات/ م^2) في كل موعد من مواعيد الشتل.

المؤشرات المدروسة:

- قراءات المجموع الخضري: أُخذت عند اكتمال النم و الخضري وقبل بدء تشكُّل الأبصال بمعدل 10 نباتات لكل معاملة ولكل مكرر:
- متوسط ارتفاع النبات (سم): حُدِّدت بقياس المسافة بدءًا من سطح التربة حتى نهاية أطول ورقة.
- متوسط عرض الورقة (سم): حُدِّدت عند أعرض منطقة من نصل الورقة الوسطى.
- متوسط عدد الأوراق/نبات.
- المساحة الورقية (سم^2): حُسبت المساحة الورقية وف ق معادلة (Gamiely et al., 1991) الخاصة بورقة البصل الأنبوبية:

$$A= (-93.1+1.83\ L+38.6\ C25)*N$$

A: المساحة الورقية/سم^2، L: طول الورقة/س م، C25: محيط الورقة على مسافة 25% من قاع دتها، N: ع دد الأوراق على النبات الواحد.

- مؤشرات الإنتاج: أُخذت هذه القـ راءات بع د جف اف الأبصال بمعدل 10 أبصال جافة في ك ل مك رر ولك ل معاملة:
- متوسط قطر البصلة الجافة (سم): بقياس القطر بين أبعد نقطتين باستخدام البياكوليس.
- متوسط وزن البصلة الجاف/غ.
- متوسط إنتاجية وحدة المساحة م ن الأبص ال الجاف ة (كغ/م^2).
- المحتوى الكيميائي للأبصال: ق ام الب احثون بتحلي ل الأبصال الجافة من حيث محتواها الكيميائي م ن النس بة المئوية للمادة الجافة%: وذلك بأخذ عدة قطع من الأبصال لكل معاملة، ثم توزن وتُجفَّف في فرن كهربـ ائي بدرج ة حرارة 110 °م حتى ثبات الوزن، وبع د انتهـ اء فت رة التجفيف يُحسب وزنها، ومن ثم تطبَّق المعادلة الآتية حسب (AOAC، 2008):

$$DM\ \%= (W1/W2)*100$$

DM: النسبة المئوية للمادة الجافة.

W1: الوزن بعد تجفيف الأبص ال، W2: الـ وزن قب ل تجفيف الأبصال.

تصميم التجربة والتحليل الإحصائي:

نُفِّذت التجربة كتجربة عاملي ة بع املين وف ق تص ميم القطاعات العشوائية الكاملة، وبأربعة مكررات لكل معاملة، العامل الأول: هو الكثافات الزراعية (3 كثافات) والعام ل الثاني: مواعيد الشتل (3 مواعيد)، وتم تحلي ل البيان ات وحساب معنوي ة الف روق والمقارن ة ب ين متوس طات المعاملات المدروسة باستخدام قيمة أق ل ف رق معن وي L.S.D عند مستوى ثقة 5% للقـ راءات الحقلي ة وعن د مستوى 1% بحالة القراءات المخبرية باس تخدام برن امج GenStat 12th.

النتائج

تأثير موعد الزراعة والكثافة النباتية في مؤشرات النمو الخضري لدى صنف البصل الأحمر المحلي:

- ارتفاع النبات: تبيّن المعطيات الواردة في الجدول (1) أن موعد الزراعة- وبغض النظر عن الكثافة النباتية- ساهمت بشكل إيجابي في زيادة ارتفاع نباتات البصل، فقد تفوَّق موعد الزراعة الأول بمتوسط ارتفاع نباتاته (68.69 سم) وبفروق معنوية على موعد الزراعة الثاني (66.44 سم) والذي تفوق معنويًا على الموعد الثالث (62.48 سم). كما أن الكثافة النباتية بين النباتات (14 نبات/م^2) أدَّت إلى ازدياد معنوي في متوسط ارتفاع النبات (69.15 سم) وبفروق معنوية على الكثافة (20 نبات/م^2) (66.25 سم) والتي تفوقت بدورها معنويًا على الكثافة (40 نبات/م^2) (62.22 سم). أما فيما يتعلق بالتفاعل بين موعد الزراعة والكثافة النباتية فكان معنويًا في صفة ارتفاع النبات، حيث تفوَّقت النباتات المزروعة في الموعد الأول عند الكثافة 14 نبات/م^2 في ارتفاع نباتاتها (73.67 سم) وبفروق معنوية على المعاملات كافة باستثناء النباتات المزروعة في الموعد الأول عند الكثافة 20 نبات/م^2 والموعد الثاني عند الكثافة 14 نبات/م^2 حيث لم تكن الفروق معنوية بينهم.

جدول (1). تأثير موعد الزراعة والكثافة النباتية في ارتفاع النباتات (سم) لدى صنف البصل الأحمر المحلي

كثافة نبات/م^2	الموعد الأول	الموعد الثاني	الموعد الثالث	المتوسط
كثافة 40 نبات/م2	64.44^{bcd}	62.44^{cd}	60.56^{d}	62.22^{c}
كثافة 20 نبات/م2	69.33^{ab}	67.00^{bc}	63.00^{bcd}	66.25^{b}
كثافة 14 نبات/م2	73.67^{a}	69.30^{ab}	63.11^{bcd}	69.15^{a}
المتوسط	68.69^{a}	66.44^{b}	62.48^{c}	
LSD 5%	المواعيد 2.212	الكثافات2.212	المواعيد * الكثافات 3.832	
CV%		5		

Means having the same letters in a column were not significantly different at p<0.05

عدد الأوراق/النبات:

تبيِّن النتائج الواردة في الجدول (2) أن مواعيد الزراعة قد ساهمت في زيادة عدد الأوراق على النبات، حيث تفوقت النباتات المزروعة في الموعد الأول بمتوسط عدد أوراقها (7.89 ورقة/نبات) معنويًا على الموعد الثاني (7.16 ورقة/نبات) والذي تفوق معنويًا على الموعد الثالث (6.62 ورقة/نبات). كما تفوقت النباتات المزروعة على الكثافتين 14 و20 نبات/م^2 بمتوسط عدد الأوراق (7.66، 7.27 ورقة/نبات، على التوالي) معنويًا على النباتات المزروعة على الكثافة 40 نبات/م2 (6.74 ورقة/نبات). أما بالنسبة للتداخل بين موعد الزراعة والكثافة النباتية فقد كان معنويًا في صفة عدد الأوراق/النبات، حيث تفوقت النباتات المزروعة في الموعد الأول عند الكثافة 14 نبات/م^2 في متوسط عدد أوراقها (8.55 ورقة/النبات) وبفروق معنوية على المعاملات كافة، باستثناء النباتات المزروعة في الموعد الأول بالكثافة 20 نبات/م2 والموعد الثاني بالكثافة 14 نبات/م^2 حيث لم تكن الفروق معنوية بينهم.

جدول (2). تأثير موعد الزراعة والكثافة النباتية في عدد الأوراق على النبات (ورقة/نبات) لدى صنف البصل الأحمر المحلي

كثافة نبات/م^2	الموعد الأول	الموعد الثاني	الموعد الثالث	المتوسط
كثافة 40 نبات/م2	6.89^{bc}	6.78^{bc}	6.20^{c}	6.74^{b}
كثافة 20 نبات/م2	7.53^{ab}	7.13^{bc}	6.80^{bc}	7.27^{a}
كثافة 14 نبات/م2	8.55^{a}	7.91^{ab}	7.21^{bc}	7.66^{a}
المتوسط	7.89^{a}	7.16^{b}	6.62^{c}	
LSD 5%	المواعيد 0.4357	الكثافات 0.436	المواعيد * الكثافات 0.754	
CV%		8.4		

Means having the same letters in a column were not significantly different at p<0.05

عرض الورقة:

تبيِّن النتائج الواردة في الجدول (3) أن موعد الزراعة الأول ساهم في زيادة عرض الورقة (1.36 سم) معنويًا على الموعد الثالث (1.19 سم) في حين لم تكن الفروق

معنوية بين الموعدين الأول والثاني.

جدول (3). تأثير موعد الزراعة والكثافة النباتية في عرض الورقة (سم) لدى صنف البصل الأحمر المحلي

كثافة نبات/م^2	الموعد الأول	الموعد الثاني	الموعد الثالث	المتوسط
كثافة40 نبات/م^2	1.25^{ab}	1.20^{ab}	1.12^{b}	1.18^{b}
كثافة 20 نبات/م^2	1.35^{ab}	1.30^{ab}	1.18^{ab}	1.29^{ab}
كثافة 14 نبات/م^2	1.48^{a}	1.36^{ab}	1.23^{ab}	1.36^{a}
المتوسط	1.36^{a}	1.26^{ab}	1.19^{b}	
LSD 5%	المواعيد 0.1093	الكثافات 0.109	المواعيد* الكثافات 0.1892	
CV%	5.7			

Means having the same letters in a column were not significantly different at p<0.05

وبالمقارنة بين الكثافات الزراعية نجد أن الزراعة على كثافة 14 نبات/م2 تفوقت معنويًا بمتوسط عرض الورقة (1.36 سم) على الكثافة 40 نبات/م^2 (1.18 سم) ولم تكن الفروق معنوية عند الزراعة على الكثافتين 14 و20 نبات/م^2. ولم يكن للتداخل بين مواعيد الزراعة وكثافات الزراعة تأثير معنوي في صفة عرض الورقة على النبات.

المساحة الورقية:

تعد المساحة الورقية مؤشرًا لحجم نظام التمثيل الضوئي، وهي المصدر الرئيس للمادة الجافة، لذا فإن علاقتها وثيقة بصفات النمو. وقد بيَّنت النتائج المتحصل عليها في الجدول (4) وجود زيادة معنوية في المساحة الورقية مع الزراعة في الموعد الأول (381.6 سم^2) وبفروق معنوية مع الموعدين الثاني والثالث، ولم تكن الفروق بينهما معنوية، كما ازدادت المساحة الورقية للنباتات المزروعة على كثافة 14نبات/م^2 (371.6 سم^2) وبفروق معنوية مقارنة مع الزراعة على كثافة 40 نبات/م^2 (287.3 سم2)، بينما لم تكن الفروق معنوية عند الزراعة على الكثافة 14 و20 نبات/م^2 (335.6 سم2).

أما التفاعل بين مواعيد الزراعة والكثافات الزراعية فقد كان له تأثيرٌ معنويٌّ في زيادة المساحة الورقية، حيث تفوقت النباتات المزروعة في الموعد الأول وعلى كثافة 14 نبات/م^2 في مساحتها الورقية (446.5 سم^2) وبفروق معنوية على المعاملات كافة، باستثناء النباتات المزروعة في الموعد الأول والثاني عند كثافة 20 نبات/م^2 (356.8، 333.0 سم^2) والنباتات المزروعة في الموعد الثاني وبكثافة 14 نبات/م^2 (378.9 سم^2)، وأعطت النباتات المزروعة في الموعد الثالث على الكثافة 40 نبات/م^2 المساحة الورقية الأقل (252.9 سم^2).

جدول (4). تأثير موعد الزراعة والكثافة النباتية في المساحة الورقية (سم^2) لدى صنف البصل الأحمر المحلي

كثافة نبات/م^2	الموعد الأول	الموعد الثاني	الموعد الثالث	المتوسط
كثافة 40 نبات/م2	311.6^{bc}	294.9^{bc}	252.9^{c}	287.3^{b}
كثافة 20 نبات/م2	356.8_{abc}	333.0^{abc}	289.4^{bc}	335.6^{a}
كثافة 14 نبات/م2	446.5^{a}	378.9^{ab}	319.4^{bc}	371.6^{a}
المتوسط	381.6^{a}	326.4^{b}	286.5^{b}	
LSD 5%	المواعيد 41.74	الكثافات 41.74	المواعيد * الكثافات 72.30	
CV%	10.5			

Means having the same letters in a column were no significantly different at p<0.05

تأثير موعد الزراعة والكثافة النباتية في مؤشرات الإنتاجية لدى صنف البصل الأحمر المحلي:

– قطر البصلة: تباينت مواعيد الزراعة فيما بينها بهذا المؤشر الجدول (5)، فقد تفوق موعد الزراعة الأول في صفة قطر البصلة (7.87 سم) وبفروق معنوية على الموعد الثاني (7.46 سم) والذي تفوق بدوره معنويًا على الموعد الثالث (6.88 سم)، وبالمقابل تفوقت صفة قطر البصلة عند الزراعة على الكثافتين 14 و20 نبات/م^2 (7.74، 7.62 سم) وبفروق معنوية على الكثافة 40 نبات/م^2 (6.86 سم). وبمقارنة التأثير المتبادل بين موعد الزراعة والكثافة النباتية، يلاحظ من الجدول أن التفاعل كان معنويًا، فقد تفوق الموعد الأول عند الكثافة 14

نبات/م^2 بمتوسط قطر البصلة (8.21 سم) وبفروق معنوية على كل المعاملات ماعدا موعد الزراعة الأول عند الكثافة 20 نبات/م^2 وموعد الزراعة الثاني عند الكثافة 14 نبات/م^2 (8.03، 7.76 سم، على التوالي) حيث لم تكن الفروق معنوية.

جدول: (5). تأثير موعد الزراعة والكثافة النباتية في قطر البصلة (سم) لدى صنف البصل الأحمر المحلي

كثافة نبات/م^2	الموعد الأول	الموعد الثاني	الموعد الثالث	المتوسط
كثافة 40 نبات/م^2	7.36^{cde}	6.99^{e}	6.23 ^{f}	6.86^{b}
كثافة 20 نبات/م^2	8.03^{ab}	7.64^{bcd}	7.17 ^{de}	7.62 ^{a}
كثافة 14 نبات/م^2	8.21^{a}	7.76^{abc}	7.24^{cde}	7.74^{a}
المتوسط	7.87^{a}	7.46^{b}	6.88 ^{c}	
LSD 5%	المواعيد 0.1929	الكثافات 0.192	المواعيد * الكثافات 0.3340	
CV%	4.8			

Means having the same letters in a column were not significantly different at p<0.05

وزن البصلة:

يعد مؤشر وزن البصلة من أهم المؤشرات الإنتاجية، ونستنتج من المعطيات الواردة في الجدول (6) تفوق الموعد الأول معنويًا بمتوسط وزن البصلة (199.3 غ) على الموعد الثاني (186.9غ) والذي تفوق بدوره معنويًا على الموعد الثالث (154.4 غ). كما ازداد وزن البصلة معنويًا عند الزراعة على الكثافة 14 نبات/م2 (238.1 غ) والذي تفوق بدوره معنويًا على الكثافة 40 و20 نبات/م^2 (112.0، 190.6 غ) والفروق بينهما معنوية، أما بالنسبة إلى التأثير المتبادل بين العاملين المدروسين فقد كان تأثيرًا معنويًا، إذ تفوق وزن البصلة للنباتات المزروعة في الموعد الأول وبكثافة 14 نبات/م^2 (267.5 غ) معنويًا على المعاملات كافة، باستثناء المعاملة المزروعة على كثافة 20 نبات/م^2 وللموعد نفسه (256.3 غ)، في حين أعطت معاملة الزراعة بالموعد الثالث بكثافة 40 نبات/م^2 أقل متوسط لوزن البصلة (100.00 غ).

جدول: (6). تأثير موعد الزراعة والكثافة النباتية في وزن البصلة (غ) لدى صنف البصل الأحمر المحلي

كثافة نبات/م^2	الموعد الأول	الموعد الثاني	الموعد الثالث	المتوسط
كثافة 40 نبات/م^2	190.5^{bc}	172.8^{c}	100.0^{e}	112.0^{c}
كثافة 20 نبات/م^2	256.3^{a}	190.0^{bc}	114.4^{de}	190.6^{b}
كثافة 14 نبات/م^2	267.5^{a}	208.9^{b}	121.4^{d}	238.1^{a}
المتوسط	199.3^{a}	186.9^{b}	154.4^{c}	
LSD 5%	المواعيد 11.28	الكثافات 11.28	المواعيد * الكثافات 19.53	
CV%	11.5			

Means having the same letters in a column were not significantly different at p<0.05

الإنتاجية في وحدة المساحة:

تعكس الإنتاجية في وحدة المساحة الدور المهم لمواعيد الزراعة المدروسة، فقد أشارت النتائج في الجدول (7) إلى وجود زيادة معنوية في متوسط إنتاجية وحدة المساحة من الأبصال الجافة عند الزراعة في الموعد الأول (2130 كغ/م^2) وبفروق معنوية على الموعد الثاني (1994كغ/م^2) والذي تفوق بدوره معنويًا على الثالث (1687 كغ/م^2)، كما ازداد متوسط الإنتاجية معنويًا عند الزراعة على كثافة 40 نبات/م^2 بين النباتات (2239 كغ/م^2) مقارنةً مع الزراعة على المسافتين 20 و14 نبات/م2 (1906، 1667كغ/م^2). وبدراسة التأثير المتبادل بين العاملين المدروسين نجد أنه معنوي، حيث تفوقت الإنتاجية عند الزراعة في الموعد الأول عند الكثافة 40 نبات/م2 (2428 كغ/م^2) وبفروق معنوية على كل المعاملات، باستثناء معاملة الزراعة في الموعد الأول عند المسافة 20 نبات/م^2 (2089 كغ/م^2) والزراعة عند الموعد الثاني بكثافة 40 نبات/م^2 (2289 كغ/م^2)، في حين كانت الإنتاجية الأقل عند الزراعة في الموعد الثالث على الكثافة 14 نبات/م^2 (1333 كغ/م^2).

جدول: (7). تأثير موعد الزراعة والكثافة النباتية في إنتاجية وحدة المساحة (كغ/م^2) لدى صنف البصل الأحمر المحلي

كثافة نبات/م^2	الموعد الأول	الموعد الثاني	الموعد الثالث	المتوسط
كثافة 40 نبات/م^2	2428^{a}	2289^{ab}	2000^{bc}	2239^{a}
كثافة 20 نبات/م^2	2089^{abc}	1900^{c}	1728^{c}	1906^{b}
كثافة 14 نبات/م^2	1872^{c}	1794^{c}	1333^{d}	1667^{c}
المتوسط	2130^{a}	1994^{b}	1687^{c}	
LSD 5%	المواعيد 127.7	الكثافات 127.7	المواعيد * الكثافات 221.2	
CV%	12.1			

Means having the same letters in a column were not significantly different at p<0.05

النسبة المئوية للمادة الجافة:

توضِّح المعطيات الواردة في الجدول (8) أن محتوى الأبصال من المادة الجافة قد ازداد عند الزراعة في الموعد الأول والثاني (22.85، 22.59%، على التوالي) وبفروق معنوية على موعد الزراعة الثالث (21.94%). كما ازداد محتوى الأبصال من المادة الجافة عند الزراعة على الكثافتين 14 و20 نبات/م^2 (22.85، 22.59%) وبفروق معنوي على الكثافة 40 نبات/م^2 (21.94%)، التداخل بين مواعيد الزراعة وكثافات الزراعة لم يكن معنويًّا في نسبة المادة الجافة.

جدول: (8). تأثير موعد الزراعة والكثافة النباتية في نسبة المادة الجافة (%) لدى صنف البصل الأحمر المحلي

كثافة نبات/م^2	الموعد الأول	الموعد الثاني	الموعد الثالث	المتوسط
كثافة 40 نبات/م^2	23.09 ^{a}	22.97 ^{a}	19.78 ^{b}	21.94 ^{b}
كثافة 20 نبات/م^2	23.85 ^{a}	22.87 ^{a}	21.04 ^{b}	22.59 ^{a}
كثافة 14 نبات/م^2	24.03 ^{a}	23.91^{a}	20.61 ^{b}	22.85 ^{a}
المتوسط	22.85 ^{a}	22.59 ^{a}	21.94 ^{b}	
LSD 1%	المواعيد 0.5168	الكثافات 0.5168	المواعيد * الكثافات 0.8952	
CV%	3.4			

Means having the same letters in a column were not significantly different at p<0.01

المناقشة

تشير النتائج السابقة إلى تفوق موعد الزراعة الأول (30 أكتوبر الشتل في الأرض الدائمة) في مؤشرات النمو الخضري والإنتاجية والنوعية، وتتوافق هذه النتائج مع ما توصل إليه كلٌّ من (Hamma, 2013; Mehri et al., 2015; Misra et al., 2014) والذين بينوا أن الزراعة المبكرة لها دور مهم في زيادة ارتفاع النبات وعدد أوراقه، وعليه زيادة المساحة الورقية، حيث ساهم الموعد الأول 30 أكتوبر في تعرض النبات إلى ظروف مناخية من درجة الحرارة وفترة إضاءة ملائمة لتكوين نمو خضري جيد، وهو ما زاد من كفاءة المواد الغذائية المصنعة خلال عملية البناء الضوئي، والتي تستخدم في تكوين أوراق جديدة مقارنةً مع المواعيد الأخرى المدروسة والتي رافقها- وبخاصة خلال فترة تكوين الأوراق- انخفاضٌ في درجات الحرارة وقلة فترة الإضاءة (دخول فصل الشتاء) وهو ما سبَّب قلة المواد الغذائية المخصصة خلال فترة البناء الضوئي، ترتَّب عليه انخفاض في المؤشرات المدروسة، كما بيّن كلٌّ من (Aboukhadrah et al., 2017; Ashagrie et al., 2021; Dawar et al., 2007; Ibrahim, 2010; Misra et al., 2014; Patil et al., 2012; Potter et al., 1999; Ud-Deen, 2008; Verdial et al., 2001).

أن الزراعة المبكرة لها دور إيجابي في زيادة الإنتاجية، وتعطي الأبصال ذات القطر الأكبر وتزيد من النسبة المئوية للمادة الجافة في الأبصال، وربما يعزى ذلك إلى أن عدد الأوراق المتشكِّلة في موعد الزراعة المبكر أكبر من عدد الأوراق المتشكِّلة في موعد الزراعة المتأخرة، وهذه تعد مفتاحًا لعملية نمو الأبصال؛ لأنها المورد الرئيس لزيادة الإنتاجية من خلال زيادة القدرة الفعالة على عملية البناء الضوئي (Mettananda & Fordham, 1999)، فخلال عملية البناء الضوئي يُنتَج السكروز الذي سيُنقل ويُخزَّن في الأنسجة الهيكلية والتخزينية لنبات أثناء تشكُّل الأبصال، حيث يُحوَّل السكروز إلى الغلوكوز

والفركتوز، والذي بدوره يزيد من الضغط الأسموزي للمواد الذائبة في خلايا غمد الورقة، وعندها يتحرك الماء إلى الداخل، ويحدث تمدُّد للخلية وزيادة حجم الأبصال الناتجة (Brewster, 2008).

أما فيما يتعلق بالكثافة النباتية فقد بيَّنت النتائج السابقة أنه كلما ازدادت الكثافة النباتية انخفضت المساحة الغذائية المخصصة لكل نبات، وعليه تزداد المنافسة بينها على الماء والمواد الغذائية اللازمة للنمو، وهو ما أثَّر سلبًا في ارتفاع النبات وعدد الأوراق المنتجة، وتتوافق هذه النتائج مع ما وجده (Mettananda & Fordham, 1999) من أن الكثافة النباتية المنخفضة (10، 20 نبات/م^2) سبَّبت زيادة معنوية في عدد الأوراق على نبات البصل، حيث إن الكثافة الزراعية المنخفضة تقلِّل المنافسة بين النباتات وتتيح توافر الماء والعناصر الغذائية، وهو ما يؤدي إلى زيادة عدد الأوراق على النبات (Khan et al., 2003)، كما تتوافق النتائج المتحصَّل عليها مع ما بيَّنه كلٌّ من (Awas et al., 2010; Bosekeng & Coetzer, 2015; Geries et al., 2015; Islam et al., 2015; Rumpel & Felczyński, 2000; Saud et al., 2013; Shock et al., 2015) وأشاروا إلى أن عدد النباتات المزروعة في وحدة المساحة لها دور مهم وإيجابي في تحديد حجم الأبصال وقطرها والإنتاجية، فالنباتات ذات الكثافة العالية تميل إلى إنتاج إنتاجية عالية من الأبصال الصغيرة، ويعود السبب في ذلك إلى أن زيادة الكثافة النباتية تؤدي إلى زيادة عدد الشتلات المزروعة ضمن القطعة التجريبية مما يؤدي إلى انخفاض الإنتاجية، حيث إن زيادة المسافة الزراعية بين النباتات ينتج عنها زيادة في النمو الخضري، وعليه زيادة وزن البصلة وقطرها، ولكن يحدث انخفاض في الإنتاجية، ومن الممكن أن تكون الزيادة في قطر البصلة، المواد الصلبة الذائبة، والمادة الجافة عند المسافات الكبيرة بين النباتات لقلة المنافسة على المواد الغذائية والرطوبة والتي تنتج عنها أبصال ذات اقطار وأوزان مرتفعة.

استنتاج

ساهمت الزراعة في الموعد الأول 30 أكتوبر مع الكثافتين 14 أو 20 نبات/م^2 في تحسين النمو والإنتاجية والصفات النوعية لصنف البصل الأحمر المحلي واختصار دورة حياته إلى موسم واحد من خلال الحصول على الأمهات من البصل (للاستهلاك المحلي) من خلال عملية الشتل.

الأخلاقيات البحثية

هذا البحث أصيل وبإشراف الباحث الأول، وجميع البيانات أصيلة وليست مقتبسة.

ازدواجية الاهتمام: الباحثون الذين أعدوا هذه المخطوطة يؤكدون عدم وجود أي اهتمام مزدوج مع جهات عامة او خاصة.

مساهمات المؤلف: ساهم المؤلف الأول والثاني في تطوير فكرة البحث وأدواته وجمع البيانات وتحليلها، وساهم المؤلف الثالث والرابع في جمع البيانات وإعداد المخطوطة النهائية.

التمويل: تم التمويل لهذه المخطوطة من قبل الهيئة العامة للبحوث العلمية الزراعية.

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Efficacy of Aqueous Extracts of some Libyan Medicinal Plants Against *Sclerotinia sclerotiorum* In Vitro

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Abstract: The study was conducted to test the effect of aqueous extracts of the leaves of ten medicinal plants growing naturally in Al-Jabal Al-Akhdar region – northeast Libya, which include: Alhagi *(Alhagi camelrum)*, Mugwort *(Artimesia herba-alba)*, Everlasting *(Helichrysum stoechas)*, Chamomile *(Anthemis noblis)*, Stinkweed *(Peganum harmala)*, Nettle *(Urtica dioica)*, Rosemary *(Rosmarinus officinalis)*, Rue *(Ruta graveolens)*, Geranium *(Pelargonium graveolens)* and Castor *(Risinus communis)* using poisoned plate method in PSA medium against Sclerotinia sclerotiorum caused seeds' rot and seedlings damping-off. The results showed a significant difference in inhibition effectiveness in all extracts against the tested fungus. Among all plants, *H. stoechas* was the most effective (88.4%), followed by *U. dioica* (79.1%), then *P. harmala* (78.3%). Chemical detections showed that the extracts of *H. stoechas*, *P. harmala*, and *U. dioica* contained some antioxidants: phenols, flavonoids, and tannins. Based on these results application of plant extracts can be considered a beneficial strategy for controlling fungal plant diseases.

فعالية المُستخلصات المائية لبعض النباتات طبية ليبية ضد *Sclerotinia sclerotiorum* في المعمل

الكلمات المفتاحية :
نباتات طبية؛
مُستخلصات مائية؛
النمو القطري؛
Sclerotinia sclertiorum؛
ليبيا.

المستخلص : استهدفت هذه الدراسة اختبار فعالية 10 أنواع من النباتات الطبية النامية بمنطقة الجبل الأخضر– شمال شرق ليبيا، مُمثلة في الشيح، وإكليل الجبل، والحرمل، والقُ راص، والع اقول، والعطرش ان، وحشيش ة الأرنب، والخروع، والبابونج، والسذاب في صورة مُستخلصات مائية خام بطريقة الطبق المسموم ف ي الوس ط الغذائي PSA ضد النمو القُطري للفطر *Sclerotinia sclerotiorum* المسبب لعفن البذور وسقوط البا دارات. أشارت النتائج إلى تباين معنوي في التأثير التثبيطي للمُستخلصات على نمو الفطر حيث سجل مُستخلص نبا ات حشيشة الأرنب فعالية أكبر في تثبيط الفطر بنسبة 88.4% متبوعًا بنبات القُراص (79.1%) يليه الحرمل بنسبة 78.4%. بيَّن الكشف الكيميائي احتواء مُستخلصات حشيشة الأرنب، والحرمل والقراص على بعض مُضا ادات الأكسدة من الفينولات والفلافونيدات والتينينات. استنادًا إلى هذه النتائج فإن تطبيق المُستخلصات النباتية يمكن أن يعد استراتيجية مُفيدة للسيطرة على أمراض النبات الفطرية.

المقدمة

تُعد منطقة الجبل الأخضر– شمال شرق ليبيا من المناطق الغنية بالتنوع النباتي؛ إذ يصل عدد الأنواع النباتية به ذه المنطقة إلى 1100 نوع تختلف من برية وطبية وعطري ة أو في شكل أعشاب وأشجار وشـ جيرات تحتـ وي علـ ى مُركبات كيميائية كنواتج أيضية ثانوية دفاعية أو مُضـ ادة من المُمْكن أن تسهم في مُكافحـ ة المُسـ ببات المرضـ ية للمحاصيل الزراعية (دراسـ ة وتقيـ يم الغطـ اء النبـ اتي، 2005).

اتجهت الدراسات إلى استخدام النباتات الطبية والعطري ة لاحتوائها العديد من المُركبات الفعالة في مُكافحة الكائنات المُمْرضة، فضلًا عن كونها آمنـ ة وغيـ ر مُلوثـ ة للبيئـ ة

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(Schwan-Estrada & Stangarlin, 2005)، حيث اختبر (منصور وأخرون، 2012) التأثير التثبيطي للخُلاصات المائية لنباتات الريحان، الثوم، الكافور، والدفلة ضد بعض الفطريات المُمْرضة للنبات، وسجلت النتائج اختلافات معنوية في النمو القُطري النبات.

في دراسة أخرى تمكن (لاربد، 2013) من خفض النمو القُطري ومُكافحة العفن الرمادي على الأبصال المُتسبب عن الفطر *Botrytis cinerea* بعد المعاملة بالمُستخلصات المائية لنباتات الشيح، والنعناع والبردقوش، في حين لُوحظ أن مُعظم المُستخلصات المائية أعطت نتائج مُشجعة في تثبيط الفطر *S. sclertiorum* بترتيب الثوم، القرنبيط، النعناع، والبصل، حيث تفوقت مُعاملة مُستخلص النيم بنسبة تثبيط بلغت 84.4% (زوين وداوود، 2015).

ونظرًا إلى تنوع الفلورا النباتية في ليبيا أُجريت هذه الدراسة لتقييم فعالية الخُلاصات المائية لبعض النباتات النامية طبيعيًّا في منطقة الجبل الأخضر – شمال شرق ليبيا ضد النمو القُطري للفطر *S. sclerotiorum* فضلًا عن الكشف اللوني عن مُضادات الأكسدة في الخُلاصات الفعالة والتي من المُمكن أن تكون مُفيدة في إدارة أمراض النبات.

مواد وطرائق البحث

تمت عملية الجمع للنباتات الطبية والعطرية من منطقة الجبل الأخضر في عدة مواقع شملت: البيضاء، وشحات، واسلنطه، وجردس، والوسيطة، والحمامة الجنوبية (جدول 1 ، شكل1)، حيث جُمع المجموع الخضري والذي شمل الأوراق السليمة الخالية من أعراض نقص العناصر أو أي أعراض أخرى، فضلًا عن جمع الأغصان والأزهار في بعض النباتات لاستخلاص المواد الفعالة، وجُمعت النباتات وقت التزهير، وهو الوقت المناسب لجمع العينات، حيث تم جمع العينات من بدء تفتح الأزهار حتى بداية اكتمالها، ففي هذه الفترة توجد المادة الفعالة في أعلى مُعدل لها من مراحل النمو المُختلفة (اليحيى، 2007).

جرى تنظيف المجموع الخضري (الأوراق، الأغصان الصغيرة والأزهار) بغسله تحت تيار خفيف من الماء وتعقيمه سطحيًّا في محلول كلوركس تجاري مُخفف 10% وغسلهِ بالماء المُعقم مرة أخرى لإزالة آثار التعقيم. جُففت العينات في الظل بعيدًا عن أشعة الشمس، ثم سُحقت في الخلاط حتى أصبحت ناعمة ووُضعت في زُجاجيات مُغلقة بعيدًا عن الرطوبة.

جدول (1). أسماء النباتات المُستخدمة والجزء المُستعمل ومكان الجمع

الاسم العربي	الاسم الشعبي	الاسم العلمي	العائلة	الجُزء المُستعمل	مكان الجمع
الشيح الأبيض	الشيح	*Artimesia herba-alba*	المركبة	الأوراق والأغصان	جردس
إكليل الجبل	الإكليل	*Rosmarinus officinalis*	الشفوية	الأوراق	شحات
الحرمل	الحرمل	*Peganum harmala*	الغردقية	الأوراق والأغصان	الحمامة الجنوبية
القراص	الحريق	*Urtica dioica*	القراصية	الاوراق	شحات
العاقول	شوك الجمل	*Alhagi camelrum*	البقولية	الأوراق	الوسيطة
العطرشان	العطرشان	*Pelargonium graveolens*	الغرنوقية	الأوراق	شحات
حشيشة الأرنب	عشبة الأرنب	*Helichrysum stoechas*	المُركبة	الأوراق والأزهار	الوسيطة
الخروع	الخروع	*Risinus communis*	السوسنية	الأوراق	شحات
البابونج	القميلة	*Anthemis noblis*	النجمية	الأوراق والأزهار	اسلنطة
السذاب	الفيجل	*Ruta graveolens*	السذابية	الأوراق والأزهار	البيضاء

شكل (1). الشكل العام للنباتات تحت الدراسة

تجهيز المُستخلصات: خُلِط 50 جم من بودرة كل نبات مع 50 مل ماء مُقطر مُعقم بواسطة خلاط لمدة 5 دقائق، وتُرك المُعلق الناتج لمُدة 24 ساعة (Overnight) في مكان مُظلم وفي درجة حرارة المعمل (20-23م°). رُشحت الخُلاصة باستخدام عدة طبقات من الشاش للتخلُص من البقايا النباتية والألياف غير المسحوقة، ثم رُشحت مرة أخرى بواسطة ورق ترشيح نوع Whattman2 No باستخدام قمع Bukhner للتخلص من جميع الشوائب، وأُخذ الراشح ومُرِّر من خلال غشاء بكتيري 0.22 µm بواسطة مُرشح Zites وجهاز التفريغ الكهربائي (Pump)، واستُقبل المُستخلص في زُجاجات مُعقمة بُنية اللون مُحكمة الغلق، ووُضعت في الثلاجة ليكون الراشح معقمًا وجاهزًا للإضافة إلى الوسط الغذائي (Amadi et al., 2010).

مصدر الفطر المُمْرض: تم الحصول على عزلة الفطر المُمْرض *Sclerotinia sclerotiorum* من بذور فاصوليا مُصابة والتي جرى عزلها وتعريفها بمعمل أمراض النبات – قسم وقاية النبات – كلية الزراعة – جامعة عمر المختار.

طريقة الطبق المسموم Poisoned Plate Method:
اُتبعت في هذه التجربة الطريقة التي وصفها Dixit وأخرون (1974) حيث قامت الباحثة بتحضير الوسط الغذائي خلاصة آجار البطاطس والسكروز Potato Sucrose Agar (PSA) وقُسّمت في دوارق مخروطية زجاجية بمُعدل 45مل/ دورق، ثم عُقِّمت، وعندما أصبحت درجة حرارتها بحدود 45م° وقبل أن تتصلب أُضيف لها 5ملليتر من خُلاصة النباتات، ورُجت قليلًا ووُزعت تكمل مُعاملة في 3 أطباق. في مُعاملة الشاهد استُبدلت خُلاصة النبات بالماء المُقطر المُعقم. وبواسطة ثاقب فلين مُعقم لُقِّحت جميع الأطباق بأقراص مُتساوية قُطرها 5مم من حواف مزرعة الفطر *S. sclertiorum* بعمر 7 أيام ووُضع بشكل مقلوب على سطح الوسط في مُنتصف الطبق، وحُضنت الأطباق في درجة حرارة 25م°. أُخذت النتائج بعد أن غطى الفطر المُمْرض كامل مساحة الطبق في مُعاملة المُقارنة، إذ سُجل قطر النمو للفطر من خلال

قياس قُطر مُتعامد لمُستعمرة نمو الفطر، وأُخذ مُعدل القراءتين في كل طبق (Tao et al., 2011)، ومُقارنتها بمُعاملة الشاهد وحساب نسبة التثبيط وفقًا لمُعادلة (Datta et al., 2004):

نسبة التثبيط (%) = قطر النمو في طبق الشاهد – قطر النمو في طبق المُعاملة × 100 / قطر النمو في طبق الشاهد

ثانيًا: الكشف الكيميائي: اختيرت 3 نباتات فقط أعطت أعلى فعالية في تثبيط الفطر وهي الحرمل والقُراص وحشيشة الأرنب. استخدمت عينات من الخُلاصات الخام (شكل2) للكشف عن بعض المكونات الكيميائية النباتية الأساسية أو الأولية التي يُحتمل وجودها في النباتات تحت الدراسة. اُعتمدت وسائل الكشف اللونية وفقًا للطريقة التي ذكرها (Apsara, 2012).

شكل (2). المُستخلصات المائية الخام للنباتات المختبرة

الكشف عن القلويدات (Alkaloids): أُضيفت نقاط قليلة من حمض البكريك ($C_6H_3N_3O_7$) تركيز 0.1% إلى 5 مل من خُلاصة النبات في أنبوبة اختبار، تكوُّن اللون الأصفر إشارة على وجود الـ Alkaloids.

الكشف عن الأنثراكوينات (Anthraquinones): أُضيف 2 مل من الكلوروفورم ($CHCl_3$) إلى 1 مل من خُلاصة النبات في أنبوبة اختبار مع الرَّج باستخدام Voryex mixer متبوعًا بالترشيح. رُجَّ الراشح مرة أخرى في وجود كمية مُساوية له من محلول الأمونيا 10%. ظهور لون وردي مُشع دليل على وجود الـ Anthraquinones.

الكشف عن الفلوفونيدات (Flavonoids): في أنبوبة اختبار أُضيف محلول الأمونيا إلى خُلاصة النبات بنسبة 1:5 متبوعًا بإضافة 1 مل من حمض الكبريتيك المُركز (H_2SO_4). ظهور لون أصفر واختفاؤه إشارة على وجود الـ Flavonoids.

الكشف عن الجليكوسيدات (Glycosides): أُضيف 5 مل من خُلاصة النبات إلى 2 مل من حمض الخليك الثلجي متبوعًا بإضافة نقطة واحدة من محلول كلوريد الحديديك ($FeCl_3$) و1 مل من حمض الكبريتيك المُركز. تكوُّن حلقة ذات لون بني على الوجه الداخلي دلالة على وجود الـ Glycosides.

الكشف عن الفينولات (Phenols): وُضع 0.5 مل من خُلاصة النبات في أنبوبة اختبار، أُضيف إليها نقاط قليلة من محلول كلوريد الحديديك ($FeCl_3$) تركيز 0.5%. تكوُّن لون أخضر داكن إشارة على وجود المُركبات الفينولية.

الكشف عن الفلوبتينات (Phlobatanins): أُضيف 1مل من حمض الهيدروكلوريك (HCL) تركيز 1% إلى 5 مل من خُلاصة النبات. غلي المخلوط في حمام مائي حتى ظهور راسب أحمر دليل وجود الPhlobatanins .

الكشف عن الستيرويدات (Steroids): أُضيف 2 مل من الخلات اللامائية إلى 0.5 مل من خُلاصة النبات، ثم أُضيف 2 مل من حمض الكبريتيك (H_2SO_4). تغيُّر اللون من البنفسجي الى الأزرق أو الأخضر إشارة على وجود الـ Steroids .

الكشف عن التينينات (Tannins): خُلط 5 مل من الماء المُقطر مع 1 مل من خُلاصة النبات ونُقلت إلى حمام مائي حتى الغليان، ثم بُرِّد الخليط، وأُضيفت إليه قطرات قليلة من محلول كبريتات الحديديك تركيز 0.1% تدريجيًّا حتى

ظهور لون بني مُخضر أو أسود مُزْرق وهو مؤشر على وجود الTannins .

الكشف عن التربينات (Terpenoids): وُضع 5 مل من خُلاصة النبات و 2 مل من الكلوروفورم في أنبوبة اختبار أُضيف لها تدريجيًّا 3 مل من حمض الكبريتيك المُركز حتى تكوُّن طبقة بنية مُحمرة إشارة على وجود الـ Terpenoids.

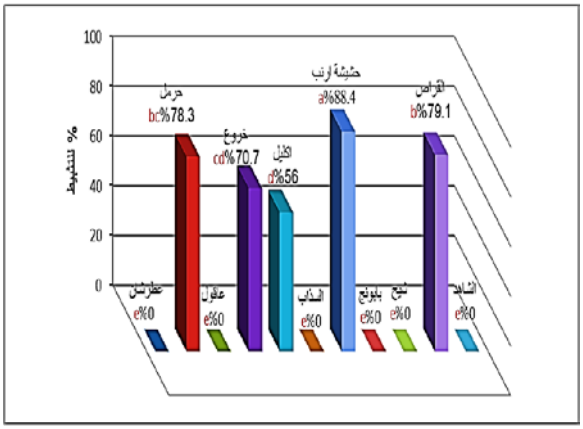
الكشف عن السابونينات (Saponins): رُجَّ 5 ملليتر من المُستخلص المائي لمدة دقيقة في أنبوبة اختبار حتى ظهور رغوة كثيفة دامت لمدة 15 دقيقة دليل على وجود الـ Saponins (Edeoga et al., 2005).

الكشف عن الراتنج (Resins): أُضيف 5 مل من الهكسان (C_5H_{10}) إلى 0.1 جم من بودرة النبات متبوعًا بإضافة الكمية نفسها من محلول أسيتات النحاس مع الرج جيدًا، ثم تُرك المخلوط حتى تنفصل الطبقات. ظهور لون أخضر دليل على وجود مواد راتنجية Resins (Ewansiha et al., 2016).

التحليل الإحصائي نُفِّذت التجربة باستخدام التصميم العشوائي التام في تحليل أحادي الجهة. النسب المئوية حُوِّلت زاويًّا من جداول Percentage Angle = $\text{Arcsin}\sqrt{\text{Percentage}}$ قبل تحليلها إحصائيًّا باستخدام برنامج Co Stat واختبار LSD تحت مستوى المعنوية ($P \geq 0.05$) للمُقارنة بين مُتوسطات المُعاملات.

النتائج

استهدفت التجربة اختبار تأثير المُستخلصات المائية للنباتات تحت الدراسة بطريقة الطبق المسموم ضد النمو القطري لميسليوم الفطر *S. sclerotiorum* في الوسط الغذائي PSA. أشارت النتائج إلى وجود اختلاف في فعالية المُستخلصات النباتات وتثبيطها لنمو الفطر (شكل 3) مُقارنة بالشاهد، حيث أظهرت مُستخلصات الحرمل، الخروع، إكليل الجبل، حشيشة الأرنب وأخيرًا القُراص تثبيطًا بدرجات مُختلفة، في حين تساوت مُستخلصات العطرشان، العاقول، السذاب، البابونج، الشيح في عدم نشاطها ضد الفطر ومُطابقتها للشاهد. أعطت نتيجة المُعاملة بمُستخلص حشيشة الأرنب أعلى نسبة تثبيط 88.4%، يليها 79.1% و 78.3% ناتجة عن المُعاملة بخُلاصة القُراص والحرمل على التوالي، ومتبوعة بمُستخلصي الخروع والإكليل اللذين سجلا نسبة تثبيط في نمو الفطر بلغت 70.7. و56.0% على الترتيب، فيما كانت باقي المُستخلصات غير فعالة ضد الفطر. نتائج التحليل الإحصائي بيَّنت وجود فروقات معنوية بين المُستخلصات في تأثيرها على النمو القُطري للفطر.



شكل (3). تباين فعالية المُستخلصات النباتية في تثبيط نمو الفطر *S. sclerotiorum* بطريقة الطبق المسموم
الأعمدة المتبوعة بالحرف نفسه تُشير إلى عدم وجود فروق معنوية عند فصل المتوسطات تحت مستوى المعنوية ($P \geq 0.05$).

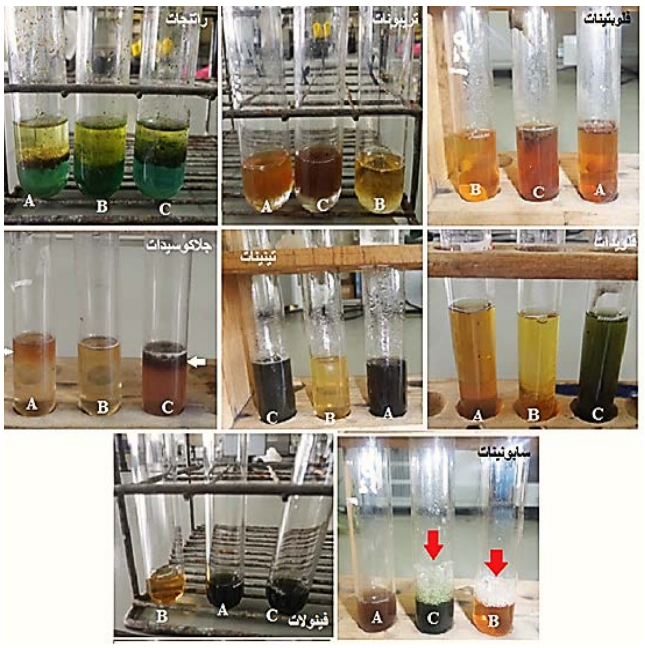
الكشف الكيميائي: أعطت المركبات الكيميائية في الخُلاصة المائية للنباتات استجابات مُختلفة أثناء الكشوفات الأولية تمثلت في ظهور ألوان مُختلفة كالأخضر للدلالة على المواد الراتنجية، الأخضر الداكن إشارة إلى الفينولات، اللون البُني المُخضر إشارة إلى وجود التينينات، ورغوة الصابون دليل وجود السابونينات (شكل 4). بيَّنت الاختبارات اللونية للكشف عن المُركبات الكيميائية في مُستخلصات النباتات المُختبرة المُوضحة في الجدول (2) وجودها بشكل مُتفاوت باختلاف أنواع النباتات، حيث

سُجِّلت القلويدات (Alkaloids) في نبات الحرمل فقط، واحتوى القُراص وحشيشة الأرنب على الجلايكوسيدات (Glycosides)، الفينولات (Phenols)، التينينات (Tannins) والتريبونات (Terpenoids)، وثبت وجود الراتنجات (Resins) في جميع المُستخلصات، في حين ظهرت السابونينات (Saponins) في الحرمل والقُراص فقط (شكل4- سهم أحمر).

جدول (2). الكيميائيات النباتية في الخُلاصات المائية الخام

المُركبات الكيميائية	الحرمل	القُراص	حشيشة الأرنب
قلويدات	+	−	−
أنثركوينات	−	−	−
فلافونويدات	−	−	+
جلايكوسيدات	−	+	+
فينولات	−	+	+
فلوتبينات	−	−	−
سابونينات	+	+	−
تينينات	−	+	+
تريبونات	−	+	+
راتنجات	−	+	+

+: دليل وجود المُركب الكيميائي
−: دليل عدم وجود المُركب الكيميائي



شكل (4). اختلاف الألوان ودرجاتها باختلاف المُركب الكيميائي في المُستخلص النباتي.
A: حشيشة الأرنب، B: الحرمل و C: القُراص. (لاحظ رغوة السابونينات عند السهم)

المُناقشة

استخدمت الدراسة 10 نباتات تنمو محليًّا تمثلت في الخروع، والسذاب، والحرمل، والبابونج، والإكليل، والقُراص، والعاقول، وحشيشة الأرنب، والعطرشان، والشيح والتي جُفِّفت وجرى استخلاصها مائيًّا، واختبارها بطريقة الطبق المسموم ضد الفطر *S. sclerotiorum* في الوسط الغذائي PSA، وأوضحت النتائج ظهور اختلافات معنوية في تثبيط الفطر بدرجات مُختلفة، وكانت مُستخلصات حشيشة الأرنب، والحرمل والقُراص أكثرها فعالية، يليها الخروع والإكليل، فيما كانت مُستخلصات العطرشان، والعاقول، والسذاب، والبابونج والشيح غير فعالة. تطابقت نتائج هذه الدراسة مع نتائج دراسات عديدة أشارت إلى اختلاف درجة تثبيط الفطر باختلاف المُستخلص المُستخدم (Dellavalle et al., 2011; Farooq et al., 2010; Masih et al., 2014؛ زوين وداود، 2015)، ويعود ذلك- ربما- إلى اختلاف كمية المواد الفعالة في مُستخلص كل نبات عن الآخر، كما أن اختلاف مناطق التثبيط بين المُستخلصات ربما يعود إلى سُمك الخيط الفطري الذي يؤدي دورًا واضحًا في التأثر بالمواد الفعالة، علاوة على أن ازدياد المساحة السطحية للخيوط الفطرية قد يؤدي إلى تأثرها أثناء امتصاص المواد المُثبطة في المُستخلص، لذا فإنها تثبط نموها (Moss, 1986)، أو قد يعود التأثير إلى انتشار بعض المواد السامة والمُثبطة للفطر في الوسط الغذائي لمنع النمو، وبناءً عليه إنتاج الأجسام الحجرية، كما ثبت تأثير المُستخلصات على البروتين، الكربوهيدرات، والدهون في الغشاء البلازمي لخلية الفطر ونفاذيته، وأيضًا تؤثر على مُستقبلات الإنزيمات النووية (Rani et al., 2006).

أكدت النتائج المُتحصل عليها في هذه الدراسة فعالية نبات حشيشة الأرنب يليه نبات القُراص، وهذا ربما يعود إلى احتوائها على مركبات كيميائية تطورت كمُضادات فطرية. هذه النباتات قد تكون تكيفت بيئيًّا لمُقاومة العدوى الفطرية، وبمعنى أخر لديها أيضًا ثانوي وهو المعروف

بالفايتوااكسينات المُضاد والمُقاوم لهجوم الفطريات بسبب تعرضها الثابت للفطريات المُتعايشة مع النبات المحصولي (Eloff et al., 2007)، وأشارت دراسات عديدة إلى أن النباتات التابعة للجنس *Helichrysum* تنتج مواد أيضية ثانوية وزيوت تعمل كمُضادات فيروسية، مُضادات فطرية، مُضادات ميكروبية (Bigović et al., 2017; Sobhy & El-Feky, 2007; Tomás-Barberán et al., 1990).

أثبت الكشف الكيميائي اللوني على المواد الفعالة في مُستخلص حشيشة الأرنب احتواءها مُركبات الفينولات، الفلافونيدات، الجلايكوسيدات، التينينات، التريبونات وهي مركبات لها قدرة عالية على الذوبان في الماء (Akrout et al., 2012)، حيث تعمل حشيشة الأرنب مُضادًا قويًا للفطر والتي ربما ترتبط بوجود مُركبات الفلافونيدات المعروفة بتضادها القوي للفطريات والبكتيريا (Cushnie & Lamb, 2005; Saravanakumar et al., 2009).

دراسات التركيب الجُزيئي لزيت نبات حشيشة الأرنب وأصناف أخرى تابعة للجنس نفسه سجَّلت احتواء الزيت على مجموعة من المُركبات كان من أهمها: β-caryophyllene ، α-humulene، α-pinene وlimonene (Roussis et al., 2002; Sobhy & El-Feky, 2007)، ويعود فعل الفلافونيدات إلى قدرتها على التداخل مع البروتينات الخلوية وتكوين مُعقد بروتيني قابل للذوبان في الماء خارج الخلية مُكون من جدار الخلية والأغشية البلازمية للميكروب بعد تمزيقها (Batchelder, 2004; Tsuchiya et al., 1996)، كما سجلت الدراسة أيضًا احتواءها على الفلافونيدات، التينينات والفينولات، وهذا يعود إلى وجود مجموعة الهيدروكسيل المُرتبطة بمجموعة الفينول ذات العلاقة بسُمِّيَّة الميكروبات المُجهرية عن طريق الإنزيمات المؤكسدة للفينولات والتداخل مع مجموعات Sulfhydryl خلال مراحل تخليق البروتين (Arif et al., 2009)، وتعود الفعالية الحيوية للمواد الأيضية الثانوية إلى حدوث تغيرات مُورفولوجية وخلوية في الكائنات الدقيقة. هذه التغيرات يُمكن أن تُدرس عينيًا أو تحت المُجهر. تتضمن التغيرات العينية تغييرًا في لون المستعمرة وشكلها، وتغيرات في عدد الخلايا، وحجم الخلية، وشكل الخلية، وعدد التراكيب المُنتجة. فيما يتعلق بتأثير المُستخلصات خلويًا ظهرت التغيرات في صورة خلايا فارغة من المُحتويات، تثبيط تخليق DNA و RNA ، البروتين والجدار الخلوي، فضلًا عن أن المُركبات الفينولية تتداخل مع تخليق الجدار الخلوي والغشاء الخلوي وتؤدي إلى تحطمه وقتل الفطر.

استنتاج

سجَّلت الدراسة تباينًا في فعالية المُستخلصات المائية للنباتات المُستخدمة ضد الفطر المُمْرض، وكانت خُلاصات الحرمل، والقُراص وحشيشة الأرنب أكثرها كفاءة في تثبيط الفطر، وهذا يعود إلى اختلاف مُحتواها من المُركبات الكيميائية المعروفة بنشاطها التضادي للميكروبات الدقيقة، وأن إدخال نبات تقليدي واسع الانتشار كمُبيد طبيعي قد يُساهم في تقليل تكاليف استعمال المُبيدات في برامج مُكافحة أمراض النبات.

الأخلاقيات البحثية

البحث جزء من رسالة ماجستير للباحث الأول تحت إشراف الباحث الثاني، كما أن جميع البيانات والصور أصيلة وليست مُقتبسة.

ازدواجية الاهتمام: يعلن المؤلفون ان المخطوطة جزء من رسالة ماجستير.

مساهمات المؤلف: البحث مستل من رسالة ماجستير والمؤلف الثاني هو المسؤول عن النسخة النهائية لهذه المخطوطة.

التمويل: يُقر المؤلف بعدم تلقي أي تمويل لدعم العمل.

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