



EFFECT OF GRAFTING ON THE STIMULATED ROOTSTOCK (*LAGENARIA*) AND FOLIAR SPRAY WITH A COMBINATION OF PHOSPHORUS AND BORON ON THE GROWTH AND YIELD OF THE MEDICINAL PUMPKIN PLANT

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Abstract

A field experiment was conducted in one of the Units of Medicinal and Aromatic Plants fields-College of Agricultural Engineering Sciences - University of Baghdad, during the spring season 2019 on the Medicinal pumpkin plant. The research aimed to study the effect of grafting on the stimulated rootstock (*Lagenaria*) and foliar spray with a combination of phosphorus and boron on the growth and yield of the Medicinal pumpkin from the fixed oil and its components of the Phytosterol and some fatty acids. Two main factors were implemented within this study, where the grafting represented the first factor, as the grafting on the rootstock *Lagenaria* (A1), and without grafting (A2), while the second factor represented the levels of foliar spray of phosphorous (P) and boron (B) with six combinations that include control (P₀B₀), phosphorous 1000 mg. L⁻¹ and without boron (P₁B₀), boron 50 mg. L⁻¹ and without phosphorous (P₀B₁), phosphorous 1000 mg. L⁻¹ and boron 50 mg. L⁻¹ (B₁P₁), boron 100 mg. L⁻¹ and without phosphorous (P₀B₂), phosphorous 1000 mg. L⁻¹ and boron 100 mg. L⁻¹ (P₁B₂). The experimental work was carried according to nested design using randomized complete blocks design RCBD with three replicates. The results showed a significant difference in plant seed yield, seed production and fixed oil yield at treating A₁P₀B₂ with 1.77 g. Plant⁻¹, 88.9 mg. h⁻¹, 23.79 mg. h⁻¹, respectively. Moreover, the highest percentages of oil and oleic acid at treating A₁P₁B₁ was 31.52 and 68.95%, respectively, and the highest percentage of linoleic acid at treating A₂P₁B (18.22%). Finally, the highest percentage of Palmitic acid at treating A₂P₁B₀ (15.10%) and the highest percentage of sterols at the two treatments of A₁P₁B₂ and A₂P₁B₁ were 1.430 and 1.280%, respectively.

Keywords: Medicinal pumpkin, phosphorus, boron, grafting, Phytosterol, fatty acids.

Introduction

Medicinal pumpkin (*Cucurbita pepo* sp. *pepo* var. *styriaca*) is a Medicinal plant that has gained nutritional and Medicinal importance in the world, which belongs to the Cucurbitaceae family that includes about 125 sex and 825 species (Deyo and O, Mally, 2008). The original home of the Medicinal pumpkin plant is North and South Latin America and has been found to grow wildly for thousands of years in Mexico and South America (Fruhwith and Hermetter, 2007). The plant has gained Medicinal importance from its seeds that contain a high content of Medicinal fixed oil (Ardabili *et al.*, 2011). Fixed oil contains a group of steroidal compounds (phytosterol) of both preventive and curative benefits for prostate hyperplasia (Habibi *et al.*, 2011; Yousefi *et al.*, 2012; Fruhwirth and Hermetter, 2007). As well as, the positive effect of oil by increasing the beneficial cholesterol (HDL) and decreasing the harmful cholesterol (LDL) (Ali *et al.*, 1999). The importance of the oil was increased due to its high content of unsaturated fatty acids, which is about 81-85% mainly composed of Oleic acid and linoleic acid depending on environmental conditions (El-Amrani *et al.*, 2015). Furthermore, the increasing demand for the Medicinal plants, including the Medicinal pumpkin plant leads to that, several agricultural operations have been applied to expand their cultivation and increase their production, including foliar application, such as phosphorus that is one of the essential nutrients for the plant due to its direct role in most biological activities within the plant cell. In addition to it is important in forming and release energy compounds and in sugars and nucleic acids synthesis, and its effect on preserving flowers from falling and increasing the set ratio and seed yield (Al-Sahaf, 1989). Additionally, due to the

high phosphorus percentage is subject to steadiness and its low availability to the plant when adding it to the soil, the foliar application was used to reduce this problem. Numerous studies have shown the role of phosphorus in the qualitative and quantitative yield in many plants. Khalid (2012) found after sprayed anise, coriander and fennel with phosphorous and nitrogen, that the level of N3P3 gave a significant increase in the percentage of the fixed oil (5.4, 4.4, 3.7% respectively). Moreover, (Yin *et al.*, 2016) noted that the addition of five levels of phosphorus (40, 30, 20, 10, 0 mg.h⁻¹) on the soybean plant occurred a significant increase in the amount of oil and fatty acids (oleic, linoleic, linolenic) at the level of 10 kg.h⁻¹ of phosphorous. As well as, Jaafar (2004) noted that the treatment of 60 kg.h⁻¹ of P₂O₅ was superior by increasing the percentage of oil in seeds *Citrullus colocynthis* plant and reached 25.62%.

The foliar application with microelements is of great importance in increasing plant growth and its content of important Medicinal compounds. As the boron element is involved with phosphorus in the DNA and RNA synthesis, and it has a role in facilitating the transfer of sugars and increasing the effectiveness of the cell membrane. As well as, its important role in the formation of pollen tubes and increasing fertilization process (Abu Dahi and Younis 1988; Abu Nuqta and Al-Shater, 2011), and increasing the seed yield. Yadav (2016) in a study aimed to determine the effect of several levels of boron for two seasons in *Brassica juncea* L. plant, found that the level 1.5 kg.h⁻¹ achieved a significant increase in number of the capsule, capsule length, number of seeds in capsule, seed yield and the percentage of oil. Moreover, Saeed (2015) observed a significant increase in the flower stalk diameter, weight of 1000 seeds and seed yield of the sunflower plant (*Helianthus annuus* L.) when

treating a plant sprayed with boron (1% of H_3BO_3 with irrigation). Al-Halfi (2012) found a significant response to white mustard plant in increasing the yield per unit area by increasing percentage of 42.14, 56.36% compared to the control treatment when spraying with a boron concentration of 300 mg.L^{-1} and for two seasons.

Among the techniques used to increase plant growth and production is the Grafting technique by grafting on rootstocks aimed to increase the ability of the plant to absorb nutrition and water and extend the productive life of the plant, in addition to its importance in resisting pests and pathogens (Oda, 2002 and Hang *et al.*, 2005). For the importance of Medicinal pumpkin plants, in particular the prevention and treatment of prostatic hyperplasia, the study aimed to increase seed and oil production per unit area through interfering with grafting on rootstocks with the foliar application by phosphorus and boron.

Materials and Methods

A field experiment was carried out on the Medicinal pumpkin plant, where its seeds obtained from the Research Unit of Medicinal and Aromatic Plants, with the aim of studying the role of the grafting on the rootstock (*Lagenaria*). As well as, the foliar spray with a combination of phosphorus and boron in the spring season during the year 2019 in one of the experiment fields of the Unit of Medicinal and Aromatic Plants (750 m^2). The land was prepared by plowing and leveling, and then it was divided into lines, where the line is a soil shoulder with a height of 15 cm and a width of about 35 cm, and the distance between the line and the other 4 m. The drip irrigation system was set on the agricultural lines (T-type). The seedlings were prepared by planting the seeds of the Medicinal pumpkin plant with the pet moos mixed with loam soil in pots of 7 cm diameter on 27/2/2019. As for the seedlings of the rootstock (that prepared for grafting), they were planted 10 days before planting the Medicinal pumpkin plant at 17/2/2019. The grafting was carried out on the rootstock (*Lagenaria* type) using the tube grafting method after the grafted seedlings reached the appropriate size (2-3 true leaves) so that the diameter of both the rootstock and the plant to be grafted is identical (1.8-2 mm). After the success of both grafting and acclimatization of seedlings, the grafted and non-grafted seedlings were planted in the field on 18/3/2019. As eight plants were planted per experimental unit and the distance between one plant and another was 50 cm, and between line and another was 4 m, after that the drip irrigation was done.

The experiment included two factors, the first (main plot) included the grafting on the rootstock (A1) and without grafting (A2). The second factor (sub plot) included the levels of foliar spray of phosphorous (P) and boron (B) in six combinations: the control (P0B0), and phosphorous 1000 mg.L^{-1} without boron (P1B0), without phosphorous and boron 50 mg.L^{-1} (P0B1), phosphorous 1000 mg.L^{-1} and boron 50 mg.L^{-1} (B1P1), without phosphorous and boron 100 mg.L^{-1} (P0B2), phosphorous 1000 mg.L^{-1} and boron 100 mg.L^{-1} (P1B2). The experimental work was carried according to

Nested design using randomized complete blocks design RCBD with three replicates, and the number of experimental units was 36, the averages were compared according to the test of the least significant difference (L.S.D) at the 5% probability level. The foliar nutrition was done using phosphorus three times: during vegetative growth and before flowering and after fruits set. As for boron, it was sprayed twice during vegetative growth and before flowering, and boron was used in the form of boric acid. The characteristics of the study were measured, which included: oil yield (g.plant^{-1}), oil yield (kg.h^{-1}), oil percentage (%), oil was estimated by the sexhlet extractor device and the ratio of phyosterols according to what was mentioned Sabir *et al.* (2003). Furthermore, the fatty acids (oleic acid, Lenoliec acid, Palmitic acid) were estimated using a gas chromatography device (GC-2010), Shimadzu model of Japanese origin, using flame ionization detection (FID) and using a Methyl-Silicon SE-30 Capillary Column with dimensions ($30\text{m} \times 0.32\text{mm} \times 0.25\mu\text{m}$).

Results

Seed yield (g.plant^{-1})

The results of the statistical analysis in Table 1 showed that there was no significant effect of grafting on the seed yield of the Medicinal pumpkin plants. In contrast, a significant increase was achieved for this trait in the treatment of spraying with phosphorus and boron at treatment P₀B₂, which recorded the highest value of $15.63\text{ g.plant}^{-1}$, compared with the lowest yield obtained from treatment P₁B₀ (8.30 g.plant^{-1}). Moreover, it was observed from the same Table that the effect of the interaction between the grafting and the spraying treatments of phosphorous and boron was significantly in the seed yield. While it was shown that the two treatments, A₁P₀B₂ and A₁P₀B₁, had the highest increase for the seed yield (17.77 and $17.16\text{ g.plant}^{-1}$, respectively), without significant differences between them, compared to the lowest seed yield reached 5.82 g.plant^{-1} at treatment A₂P₁B₂.

Seed production (kg.h^{-1})

The results of Table 1 indicated that there were no significant differences with the effect of grafting on the seed yield characteristic, while the significant effect of the spraying treatment with different combinations of phosphorus and boron was achieved in this characteristic. As the two treatments, P₀B₂ and P₀B₁ gave the highest production of 78.2 and 66.2 kg.h^{-1} respectively, compared to the lowest yield recorded for the treatments P₁B₀ and P₁B₂ of 41.5 and 38.8 kg.h^{-1} , respectively, and without significant difference from the comparison treatment P₀B₀ (48.5 kg.h^{-1}). The significant effect in this characteristic continued to the interaction between study factors, from the Table results, it was observed that the highest seed yield was recorded in the treatments A₁P₀B₂ and A₂P₀B₁ reached 88.9 and 85.8 mg.h^{-1} , respectively, compared to the lowest yield given by treatment A₁P₁B₂ (29.1 kg.h^{-1}).

Table 1 : Effect of grafting, spraying treatments with phosphorus and boron and interaction in seed yield of the plant (g.plant⁻¹), seed yield (kg.h⁻¹) of the Medicinal pumpkin plant

Spraying treatments (mg.L ⁻¹)	Seed yield (g.plant ⁻¹)			Seed production (kg.h ⁻¹)		
	Grafted	Non-grafted	Average	Grafted	Non-grafted	Average
POB0	11.24	8.15	9.69	40.8	56.2	48.5
P1000B0	6.60	10.01	8.30	33.0	50.0	41.5
POB50	17.77	9.30	13.23	46.5	85.8	66.2
P1000B50	11.31	11.34	11.23	56.6	56.7	56.6
POB100	17.16	13.50	15.63	88.9	67.5	78.2
P1000B100	9.70	5.82	7.76	48.5	29.1	38.8
L.S.D (5%)	3.45			17.2		
Average	10.98	10.99		55.0	54.9	
L.S.D (5%)	N.S		2.44	N.S		12.2

The Percentage of fixed oil %

Table 2 showed that the grafting treatment had a significant effect on increasing the percentage of oil for Medicinal pumpkin plants, as the grafted plants achieved the highest percentage for this characteristic of 28.32% compared to non-grafted plants (26.16%). As for the effect of spraying with phosphorus and boron, it was also significant in this characteristic, as the P₁B₂ treatment achieved the highest percentage (28.75%) compared to the comparison treatment P₀B₀ (26.97%) superior over the P₀B₁ treatment which recorded the lowest percentage of 25.60%. The significant effect on the interaction treatments continued in increase the percentage of oil, as treatment A₁P₁B₁ recorded the highest percentage of oil that reached 31.52%, which significantly superior over the rest of treatments compared to the treatment A₂P₀B₀ which recorded 24.63% and did not differ significantly from the lowest value recorded at treatment A₂P₁B₁ (24.13%).

Fixed oil yield (kg.h⁻¹)

The results of the statistical analysis in Table 2 indicated that there were no significant differences in the oil yield with the effect of the grafting treatment, while the significant increase appeared at spraying treating with different levels of phosphorus and boron elements. The treatment P₀B₂ exceeded all the treatments by giving the highest oil yield of 21.75 kg.h⁻¹, while the lowest oil yields at the two treatments P₁B₀ and P₀B₀ (10.82 and 13.10 kg.h⁻¹), respectively. Furthermore, the results of the same Table showed that the oil yield was affected by the interaction treatments. As the two treatments A₁P₀B₂ and A₂P₀B₁ gave the highest oil yield reached 23.79, and 21.95 kg.h⁻¹, respectively, compared to the lowest values, which recorded at the treatments A₂P₁B₂ and A₁P₁B₀ by 8.11 and 9.12 kg.h⁻¹, respectively and without significant difference from the treatment A₂P₀B₀ that gave 10.05 kg.h⁻¹.

Table 2 : Effect of grafting, spraying treatments with phosphorus and boron and interaction in the percentage of fixed oil percentage, fixed oil yield (mg.h⁻¹) of Medicinal pumpkin Plant

Spraying treatments (mg.L ⁻¹)	Percentage of fixed oil %			Fixed oil yield (mg.h ⁻¹)		
	Grafted	Non-grafted	Average	Grafted	Non-grafted	Average
POB0	29.30	24.63	26.97	16.15	10.05	13.10
P1000B0	28.17	25.10	26.63	9.12	12.52	10.82
POB50	25.37	25.83	25.60	11.92	21.95	16.94
P1000B50	31.57	24.13	27.85	17.79	13.70	15.75
POB100	26.10	29.17	27.63	23.79	19.72	21.75
P1000B100	29.40	28.10	28.57	14.20	8.11	11.15
L.S.D(5%)	1.08			4.66		
Average	28.32	26.16		15.40	14.33	
L.S.D(5%)	0.77		0.79	N.S		3.29

The percentage of Oleic fatty acid

The results of Table 3 indicated that there were no significant differences in the percentage of oleic fatty acid with the effect of grafting treatment. Whereas the phosphorous and boron spray treatments had a significant effect in increasing the percentage of fatty acid, as the highest percentage were at treating P₁B₁ of (62.80%) compared to the lowest percentage reached 48.32% at treating P₀B₁. As for the interaction treatments, it was found that there were a significant differences between them, as the highest percentage of fatty acid in the two treatments A₁ P₁B₁ and A₁P₀B₀ were (68.95 and 68.30%, respectively), and without significant difference between them, compared with

the lowest percentage of acid recorded at the treatment A₁P₀B₁ (42.95%).

The percentage of Linoleic fatty acid

The results of Table 3 showed that the linoleic acid percentage was not affected by grafting treatment. Whereas, the spraying treatments with a combination of phosphorus and boron had a significant effect on increasing the percentage of fatty acid, as the highest percentage was at treatment P₁B₁ (15.87%), followed by the treatment P₁B₂ (15.00%), compared to the lowest percentage recorded at treatment P₀B₁ (13.38%), which did not differ significantly from the control treatment (13.72%). The same Table also

showed the effect of significant interaction between the experiment factors in increasing the percentage of fatty acid, the highest percentage was achieved at treatment $A_2P_1B_2$

reached 18.22% compared to the lowest percentage recorded at the treatment $A_1P_0B_1$ with 10.81 and 10.16%, respectively.

Table 3 : Effect of grafting, spraying treatments with phosphorus and boron and interaction in the percentage of fatty acids Oleic, Linoleic of the Medicinal pumpkin plant

Spraying treatments (mg.L ⁻¹)	Oleic			Linoleic		
	Grafted	Non-grafted	Average	Grafted	Non-grafted	Average
P0B0	68.30	49.87	59.09	16.64	10.81	13.72
P1000B0	45.07	61.42	53.25	10.17	17.51	13.84
P0B50	42.95	53.69	48.32	10.16	16.61	13.38
P1000B50	68.95	56.65	62.80	14.30	17.44	15.87
P0B100	54.66	45.67	50.16	15.14	14.14	14.64
P1000B100	55.73	51.61	53.67	11.77	18.22	15.00
L.S.D(5%)	5.37			2.09		
Average	55.94	53.15		13.03	15.79	
L.S.D(5%)	N.S		4.01	N.S		1.47

The percentage of Palmitic fatty acid

The results of Table 4 showed that there was no significant effect of the grafting treatment on the palmitic fatty acid percentage, while the spraying treatments with phosphorus and boron showed significant differences between them. As the highest percentage of fatty acid at control plants reached 14.34%, followed by the two treatments P_1B_1 and P_1B_0 without a significant difference that recorded 13.95 and 13.62%, respectively, compared to the lowest percentage (10.89%) at treatment P_1B_2 . Whereas, the effect of the interaction between the grafting treatments and the levels of spraying with phosphorus and boron, it was also significant in increasing the percentage of fatty acid. As the $A_2P_1B_0$ was significantly superior by giving the highest percentage of 15.10%, followed by $A_1P_0B_0$ and $A_1P_1B_1$ and without significant difference of 14.76 and 14.67%,

respectively, compared to the lowest percentage recorded at treatment $A_1P_0B_1$ of 10.08%.

The percentage of Phytosterols

The results of Table 4 indicated that the percentage of phytosterols was not significantly affected by the grafting treatment. While it can be observed that there are significant differences between the spraying levels with phosphorus and boron in their effect in this characteristic. As the highest percentage was recorded at treatment P_1B_1 (1.261%) compared to the lowest percentage recorded at treatments P_0B_0 and P_0B_2 (1.055 and 1.077%, respectively). The significant effect was extended in the indicated characteristic by the effect of the interaction treatments, as the highest percentage of phytosterols at the two treatments $A_1P_1B_2$ and $A_2P_1B_1$ (1.430% and 1.280%) without comparison with the lowest percentage recorded at the treatment $A_2P_1B_2$ (0.900%) and the control treatment $A_1P_0B_0$ (0.984%).

Table 4 : Effect of grafting, spraying treatments with phosphorus and boron and interaction in the percentage of palmitic, phytosterols of the Medicinal pumpkin plant

Spraying treatments (mg.L ⁻¹)	Percentage of palmitic			Percentage of phytosterols		
	Grafted	Non-grafted	Average	Grafted	Non-grafted	Average
P0B0	14.76	13.92	14.34	0.984	1.126	1.055
P1000B0	12.13	15.10	13.62	1.152	1.093	1.122
P0B50	10.08	13.48	11.78	1.224	1.116	1.170
P1000B50	14.67	13.22	13.95	1.241	1.280	1.261
P0B100	11.15	10.63	10.89	1.092	1.061	1.077
P1000B100	10.73	12.68	11.71	1.430	0.900	1.165
L.S.D(5%)	1.62			0.190		
Average	12.26	13.178		1.187	1.096	
L.S.D(5%)	N.S		1.24	N.S		0.135

Discussion

It can be observed from the previous results that the best seed yield was recorded at the treatments P_0B_1 , P_0B_2 , and P_1B_1 (Table 1) which may be due to the importance of phosphorus and boron in increasing plant efficiency and performance of metabolism. As well as, transfer its products to other plant parts such as fruits and seeds, in addition to providing nutrition during the stages of plant growth, which leads to the hormonal balance that affects the production of

flowers and its fertilization (Malik *et al.*, 1992). This explains the increase in the seed yield when a foliar application with boron may be due to its role in the growth of pollen tubes for pollen, which acts as a directed for the pollen tube during fertilization (Robbert *et al.*, 1990). As well as, (Abu Nuqta and Al-Shater, 2011) pointed out that the boron element is necessary for cell division, pollen production, fertilization and seed set. In addition, the role of boron in transporting carbohydrates to their storage locations (seeds), as the growth of the pollen tube is related to the presence of phosphorus

and boron within the cell wall components of the pollen tubes (Leonard *et al.*, 2014). On the other hand, it can be observed a decrease in the yield and seed production (Table 1) in the P₁B₂ treatment plants, which may be due to the high concentration of the two elements, which led to negative results that led to a yield decrease. Despite the superiority of the grafted plants by the oil percentage over the non-grafted plants as shown in Table 2, it can be observed that there are no significant differences in the oil yield between the grafted and non-grafted plants. Moreover, it can also be observed that most of the spraying treatments with phosphorous and boron have increased in the oil percentage or not significantly decreased compared with control treatment (except the treatment P₀B₁). This may be due to the improvement in physiological processes and biological pathways leading to the formation of oil by the effect of phosphorus and boron (Manaf *et al.*, 2017; Konuskan *et al.*, 2017). The phosphorus and boron elements interact with carbohydrates synthesis that leads to an increase in oil crops (Rehim, 2016).

In the oil yield, it can be observed the superiority of treatments P₀B₁, P₀B₂ and P₁B₁, which was due to increasing the seed yield in these treatments and increase the percentage of oil in the treatments P₁B₁ and P₀B₂ over the control. As the oil yield depends on the seed yield and the percentage of oil (Tables 1, 2).

It was found from the results in Table 3 that the treatment P₁B₁ recorded a superiority in the percentage of Oleic acid and Linoleic acid, as for the phytosterols percentage (Table 4) improved in all spraying treatments, with a significant superiority of the P₁B₁ treatment compared to the control. This may be due to the improvement in plant growth due to the effect of foliar application, which was reflected in the specific characteristics of the oil represented by fatty acids and phytosterols.

Conclusion

At grafting on stimulant rootstocks, the response depends on the type of plant to be stimulated and the rootstocks that grafted on it. Although the use of the foliar application is common with smaller nutrients, it can also be used with major important elements such as phosphorous, of which a large percentage of it is subjected to steadiness in soil. As phosphorus and boron have a mutual role in increasing the seed yield and its components in seed crops such as Medicinal pumpkin, especially the oil percentage, and oil content from phytosterols and fatty acids.

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