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Effect of Foliar Application with Kinetin and Amino Acids in the Vegetative Growth and Chemical Content of Young Olive Trees cv. "K18"

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ABSTRACT

The effect of foliar application with growth regulator of kinetin and amino acid solution under the trade name of vegeamino in the vegetative growth and chemical content of young olive trees cv. "K18" was investigated for the period from April 2020 to December 2020 in the olive orchard belonging to the Department of Horticulture and Landscape Gardening, College of Agriculture, University of Anbar, Iraq. A factorial experiment was conducted according to the Randomized Complete Block Design (RCBD), with three replicates for each treatment. The kinetin was sprayed at four concentrations (0, 50, 100 and 150 $mg.L^{-1}$) and spraying with three levels of amino acid solution (0, 1.25 and 1.50 $ml.L^{-1}$). The two study factors showed a significant effect in most of the studied growth characteristics especially the foliar spraying treatment of kinetin at a concentration of 150 $mg.L^{-1}$ and the spraying with amino acids at a concentration of 1.50 $ml.L^{-1}$ where achieved a significant superiority in the length of new branches, leaves number increment, leaf area, the percentage of dry matter in leaves, branches content of total carbohydrates, leaves content of nitrogen as well as total chlorophyll compared to the control treatment which achieved the lowest values.

Keywords: Foliar spraying, Cytokinins, Amino acids, Olives, Vegetative growth.

INTRODUCTION

Olive (*Olea europaea* L.) has been known as "the fragrance of the soft gold" due to its high economic, social and cultural values. Nowadays, olive is the most extensively cultivated oleiferous tree species in the world, covering an area of 10 million of hectares (Lanza, 2011; FAO, 2019). The olive is an evergreen tree, belongs to family Oleaceae and includes many cultivars which are used for pickling and oil extraction (Vesson, 2007). In addition, olive is well adapted to a wide range of growing conditions and soils because of its special leaf structure and ramified root system (Ben and Boukhris, 2002).

Despite the availability of all eco appropriate conditions for large areas of olive cultivation in Iraq, it has not spread widely for several reasons, including the low productivity of the tree compared to neighboring countries and the neglect of the existing orchards in addition to securing saplings suitable for cultivation as well as slow growth in the nursery and after transportation to the sustainable place comes on top of the obstacles facing the expansion of olives cultivation. This calls for the use of means to improve and accelerate growth, including foliar spraying with plant growth regulators which have a main role in different physiological processes necessary for the plant growth and development (Davies, 2004).

Kinetin is considered the most known cytokinins, where it has physiological efficacy is represented by stimulating cell division, stimulating lateral buds growth, uptake, transport and assimilation of nutrients, increasing the effectiveness and activity of apical meristem,

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promoting chloroplast development, chlorophyll biosynthesis, delaying leaf senescence, hormonal regulation of plant morphogenesis and other effects (Mok and Mok, 2001; Sakakibara, 2006), many researchers indicated to the importance of treatment with cytokinins because of their important role in enhancing plant growth traits, such as Abou Aziz et al., (2011) when spraying benzyl adenine (BA) on olive trees, El-Badawy and Abd El-Aal (2013) when spraying mango (*Mangifera indica* L.) saplings with kinetin, Muralidhara et al., (2014) when spraying benzyl adenine on mango saplings, Mahmoud et al., (2015) when spraying olive trees with benzyl adenine, Al-Janabi (2018) when spraying kinetin on cleopatra mandarin (*Citrus reshni* Hort. ex Tan.) saplings.

The plant needs amino acids mainly for its growth and development, foliar spraying of the amino acids is very necessary for plants because they considered the building blocks in the synthesis of proteins. Amino acids are organic nitrogenous compounds contain both acid and basic groups and act as buffers, which help to maintain favorable pH value within the plant cell (Davies, 1982; Hildebrandt et al., 2015). Its importance comes in its wide use where it is considered an initiator for the biosynthesis of some plant hormones (Hashimoto and Yamada, 1994; Singh, 1999). It also participates in the biosynthesis of numerous non-protein nitrogenous materials like pigments, vitamins, coenzymes as well as purine and pyrimidine bases (Bell, 2003). Amino acids directly or indirectly affect the physiological activities in plant, where it contributes to increasing the photosynthesis efficiency, stomata movement (D'Mello, 2015) and mitigating the damage caused by environmental stresses, etc. (Hammad and Ali, 2014; Rodrigues-Correa and Fett-Neto, 2019). Many researchers have indicated to the role of amino acids as biostimulants that contribute to improving the growth indicators such as Aml et al., (2011) when spraying amino nutrient (Pepton$^{85/16}$) on olive saplings, El-Shazly and Mustafa (2013) when spraying a mixture of amino acids (Amino green II) on orange (*Citrus sinensis* L. Osbeck) trees, Rasmia et al., (2014) when spraying offshoots of the date palm (*Phoenix dactylifera* L.) with several types of amino acids, Ali et al., (2019) when spraying amino nutrient on olive trees, Al-Janabi (2020) when spraying sweet orange saplings with amino acids solution (Amino Plus TG).

MATERIALS AND METHODS

This study was conducted in the olive orchard belonging to the Department of Horticulture and Landscape Gardening, College of Agriculture, University of Anbar, Iraq for the period from April 20120 to December 2020 in order to investigate the effect of foliar application with Kinetin and amino acids in some vegetative growth characteristics and chemical content of young olive trees cv. "K18". A 36 young trees were selected at the age of 6 years, uniform in their growth as much as possible planted at 5 X 5 m apart under drip irrigation, In addition, all service operations were conducted for all trees from fertilization, weeding, control of insects and diseases when needed. Four main shoots were selected distributed on the perimeter of each tree and four shoots at the age of one year were chosen for each main shoot (sixteen shoots for each experimental unit), then each shoot was marked for the purpose of making measurements of the traits that were studied, also samples from the soil were taken for the purpose of conducting some chemical and physical analysis prior to the implementation of the experiment as shown in (Table 1).

Table 1: Physical and chemical properties of the experimental soil

Particle size distribution (g.kg^{-1} soil)						**Available nutrients (mg.kg^{-1} soil)**		
Sand	**Loam**	**Clay**	**Texture**	**pH**	**EC (1:1) ds.m^{-1}**	**N**	**P**	**K**

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521.3	**272.2**	**206.5**	**Sandy loam**	**7.4**	**4.23**	**68.1**	**12.3**	**174.5**

The experiment included two factors: the first factor included foliar spraying with kinetin at four concentrations: K0 (spraying with distilled water), K1 (50 $mg.L^{-1}$), K2 (100 $mg.L^{-1}$), and K3 (150 $mg.L^{-1}$). The plant growth regulator of kinetin produced by Avonchem Co. Ltd. / UK (I.A. 99%) was prepared after the weighed of the growth regulator powder according to each concentration and dissolved in 20 ml of ethanol with a concentration of (90%) and then complete the volume to 1 liter with distilled water. The second factor included foliar spraying at three concentrations with a mixture of amino acids solution under the trade name (Vegeamino) are: A0 (spraying with distilled water), A1 (1.25 $ml.L^{-1}$) and A2 (1.50 $ml.L^{-1}$), where the amino solution is produced by Artal Agronutrientes Co. / Spain, where it contains free amino acids in the form of L-amino acids as shown in (Table 2).

Table 2: Contents of Vegeamino solution

Aspartic acid	**0.180%**	**Glycine**	**1.950%**	**Alanine**	**0.157%**
Valine	**0.017%**	**Leucine**	**0.006%**	**Proline**	**0.065%**
Glutamic acid	**16.22%**	**Threonine**	**0.039%**	**Tyrosine**	**0.147%**
Phenylalanine	**0.026%**	**Lysine**	**0.363%**	**Tryptophan**	**0.320%**
Serine	**0.316%**	**Arginine**	**0.006%**	**Methionine**	**0.012%**
Isoleucine	**0.001%**	**Histidine**	**0.108%**		

The foliar spraying process was conducted in accordance with the following dates: (3/4, 3/5, 3/6 and 3/9) by using a backpack sprayer, with capacity of (16 liters) until the drip point with the addition of a wetting agent (Triton B) to the spraying solution at a concentration of (0.1%).

A factorial experiment with two factors (4 × 3) was conducted according to the Randomized Complete Block Design (RCBD), with three replicates and each replicate represented by on tree per treatment. Data were analyzed according to the statistical program of GeneStat, and the arithmetic averages were compared by using the least significant difference test at a probability level of 0.05 (Al-Mohammedi and Al-Mohammedi, 2012).

Studied traits:

1- The Number and length of the new branches:

The number of new branches (branch. $shoot^{-1}$) was calculated and the lengths of new branches (cm) were measured on the marked shoots at the end of the experiment in December of 2020 and the average was extracted for each replicates and for each treatment.

2- Leaves number increment (leaf. $shoot^{-1}$):

The number of leaves for the marked shoots was calculated before conducting the treatments in April of 2020, the readings were taken again at the end of the experiment in December of 2020. The average increase was calculated by the difference between the two readings.

3- Leaf area (cm^2):

10 fully expanded leaves were taken from the fourth leaf to the sixth from the tip of shoots (40 leaves/ experiment unit) in December of 2020 the leaf area was calculated by using the following equation:

Leaf area (cm^2) = 0.53 (length x width) + 1.66 (Ahmed and Morsy, 1999).

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4- The percentage of dry matter in the leaves**:**

The 40 leaves were taken from the fourth to the sixth leaf from the tip of shoots in December of 2020 and washed with distilled water several times to remove the stuck from the dust and left in dry air then, weighed using a sensitive electrical balance, and dried in an electric oven at 65 ºC until the stability of weight, the percentage of dry matter was calculated according to the following equation:

Dry matter percentage in leaves = Dry sample weight (g) / Fresh sample weight (g) x 100.

5- The shoots content of total carbohydrates (%):

It was estimated according to (Dubois et al., 1956).

6- The percentage of nitrogen in leaves:

The fully expanded leaf samples were taken from the middle of the shoots at the end of the experiment and washed with distilled water several times and dried at 65°C until weight stability. Nitrogen was estimated using Microkjeldahl according to the method indicated by (Bahargava and Raghupathi, 1999).

7- The leaves content of total chlorophyll (mg.100g^{-1} fresh weight):

The fully expanded leaves were taken for extracting chlorophyll a and b, a 0.5 g fresh weight of leaves were taken and placed in tubes, a 20 ml of acetone (95%) was added to it and left for 72 h until the chlorophyll pigment was totally extracted, light absorption of the solution was determined at wavelengths (663 and 645nm) using a spectrophotometer, the leaves content of total chlorophyll was calculated according to the method mentioned by (Bajracharya, 1999).

RESULTS AND DISCUTION

1- Number of new branches (branch. shoot^{-1}) and their length (cm):

The study treatments affected the number and length of new branches. Table (3) shows that the foliar spraying with growth regulator of kinetin at a concentration of K3 has achieved a significant increase through giving the highest values amounted of 3.71 branch. shoot^{-1} and 14.95 cm while the lowest values were 2.06 branch. shoot^{-1} and 9.57 cm at the concentration of K0. The same table showed no significant differences in the new branches number due to the treatment with the amino solution (Vegeamino), while the spraying with amino acid solution affected significantly the length of new branches where the level of A2 achieved the highest value which amounted to 13.13 cm compared to the lowest value were 11.03 cm at the level of A0. The interaction between the two study factors showed a significant effect by achieving the treatment K3 X A2 the highest values for these two traits amounting to 4.60 branch. shoot^{-1} and 15.60 cm respectively, while the treatments K0 X A2 and K0 X A0 recorded the lowest values, which amounted to 1.50 branch. shoot^{-1} and 8.00 cm respectively for both traits.

The reason for the superiority of the spraying with the growth regulator of kinetin may be attributed in the increase in number and length of the new branches to its role in breaking of apical dominance and stimulating the growth of lateral buds (Bangerth et al., 2000), the increase in new branches lengths due to the growth regulator may be attributed to its role in stimulating the cellular division in apical meristems and adding new cells to the plant (Davies, 2004).These results agree with the results of El-Badawy and Abd El-Aal, (2013) which showed that the foliar spraying with the kinetin on mango seedlings had a significant effect in increasing the number and length of shoots, also these results agree with El-Sayed (2018) that the spraying of cleopatra mandarin saplings with kinetin has led to a significant increase in stem length.

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Table 3: Effect of foliar application with kinetin and amino acids and their interaction in the number and length of new branches of young olive trees

Number of new branches (branch.shoot^{-1})					Length of new branches (cm)			
Kinetin (K) mg.L^{-1}	Amino acids (A) ml.L^{-1}			Means (K)	Amino acids (A) ml.L^{-1}			Means (K)
	A0	A1	A2		A0	A1	A2	
K0	2.63	2.03	1.50	2.06	8.00	9.56	11.16	9.57
K1	2.83	2.80	2.67	2.77	9.30	11.83	12.30	11.14
K2	2.90	3.33	3.63	3.29	12.40	13.20	13.46	13.02
K3	3.13	3.40	4.60	3.71	14.43	14.83	15.60	14.95
Means (A)	2.87	2.89	3.10	Means (A)	11.03	12.35	13.13	
LSD 0.05	K	A	KXA		K	A	KXA	
	0.60	N.S.	1.04		0.54	0.47	0.95	

The increase in the lengths of new branches as a result of spraying with amino solution may be due to its content of amino acids as shown in Table (2) and its role in providing available formula for the plant from the building blocks of proteins (Davies, 1982 and Hildebrandt et al., 2015), in addition to being an important source for organic nitrogen and contributes to the construction of the secondary compounds (Tegeder, 2012). As well as their important role in metabolic activities and stimulation of many enzymes and co-enzymes and biosynthesis of some plant hormones and increase photosynthesis efficiency (Hashimoto and Yamada, 1994 and Bell, 2003 and D'Mello, 2015), which leads to the improving plant growth. These results agree with Aml et al. (2011) who found that spraying olive saplings with amino nutrient (Pepton$^{85/16}$) led to a significant increase in the plant height, also agree with Al-Janabi (2020) which showed that foliar application on sweet orange saplings with a mixture of amino acids (Amino Plus TG) led to a significant increase in shoots length.

2- Leaves number increment (leaf. shoot^{-1}) and leaf area (cm^2):

Table (4) shows that the increment of leaves number and leaf area increased as a result of treatment with kinetin, especially the K2 concentration, which had a significant effect by giving the highest values amounted to 29.30 leaf. shoot^{-1} and 3.95 cm^2, compared to the lowest values were 18.38 leaf. shoot^{-1} and 3.61 cm^2 at the K0 concentration. The same table showed significant differences as a result of spraying with an amino acids solution where the two traits increased by increasing the levels of spraying, where the A2 level achieved the highest values amounted of 28.04 leaf. shoot^{-1} and 3.90 cm^2, while the lowest values were 20.49 leaf. shoot^{-1} and 3.67 cm^2 at the A0 level. The interaction between the two study factors had a significant effect in increasing the number of leaves and leaf area, where the interaction treatment K3XA2 achieved the highest values which amounted 33.27 leaf. shoot^{-1} and 4.06 cm^2 while the treatment K0XA0 recorded the lowest values, which amounted to 15.10 leaf. shoot^1 and 3.29 cm^2 respectively for both traits.

Table 4: Effect of foliar application with kinetin and amino acids and their interaction in the leaves number increment and leaf area of young olive trees

Leaves number increment (leaf.shoot^{-1})					Leaf area (cm^2)			
Kinetin (K) mg.L^{-1}	Amino acids (A) ml.L^{-1}			Means (K)	Amino acids (A) ml.L^{-1}			Means (K)
	A0	A1	A2		A0	A1	A2	

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K0	15.10	19.80	20.23	18.38	3.29	3.76	3.77	3.61
K1	16.87	28.67	31.30	25.61	3.71	3.75	3.83	3.76
K2	22.63	24.90	27.37	24.97	3.85	3.74	3.95	3.85
K3	27.37	27.27	33.27	29.30	3.84	3.96	4.06	3.95
Means (A)	20.49	25.16	28.04	**Means (A)**	3.67	3.80	3.90	
LSD 0.05	**K**	**A**	**KXA**		**K**	**A**	**KXA**	
	3.06	2.65	5.30		0.12	0.10	0.21	

The reason for the increase in the number of leaves as well as leaf area of the olive young trees derived from treatment with kinetin may be attributed to the role of it in stimulating the growth of leaves primordia and chloroplast development, kinetin also influence the allocation of nutrients and assimilates in the plant towards treated tissues with it (Mok and Mok, 2001; Sakakibara, 2006) and their use in the building of vegetative parts. The increase in the number of leaves and leaves area may be due to the effect of the study factors in improving the traits of vegetative growth, including the number and length of shoots (Table 3), as well as improving the nutritional status, the accumulation of carbohydrates and its relationship in increasing the number of leaves and their area**.** These results are consistent with Muralidhara et al. (2014) where their results showed a significant increase in number of leaves and their area for mango saplings when foliar spraying with the benzyl adenine, also consistent with Mahmoud et al. (2015) were the leaf area significantly increased when spraying the olive trees with benzyl adenine, it also agree with Ali et al. (2019) where their results showed a significant increase in leaf area of olive trees when foliar spraying with amino nutrient, and agree with Rzouki et al. (2019) who found that spraying fig (*ficus carica* L.) saplings with amino nutrient (Terra-sorb) led to a significant increase in the leaves number and their area.

3- Percentage of dry matter in the leaves and the shoots contents of total carbohydrates (%):

Table (5) indicates a significant differences in the percentage of dry matter in the leaves and the shoots content of total carbohydrates due to the spraying with kinetin where the K2 concentration was significantly excelled on the other concentrations by giving the highest values amounted of 45.69% and 7.89% while K0 concentration showed the lowest values amounted of 44.34% and 7.32%. The foliar application with amino acids solution led to a significant increase in these two traits by increasing levels of spraying, where the level of A2 achieved the highest values, which amounted to 45.68% and 7.88%, while the lowest values were at level A0 which amounted to 44.29% and 7.31%. The interaction between the two factors showed its significant effect by achieving the treatment K3 X A2 the highest values for these two traits, which reached 46.00% and 8.30%, while the lowest values amounted to 43.00% and 7.10% at the treatment of K0 X A0.

Table 5: Effect of foliar application with kinetin and amino acids and their interaction in the percentage of dry matter in leaves and the branches content of total carbohydrates of young olive trees

Percentage of dry matter in leaves (%)					**Branches contents of total carbohydrates (%)**			
Kinetin (K) mg.L^{-1}	**Amino acids (A) ml.L^{-1}**			**Means (K)**	**Amino acids (A) ml.L^{-1}**			**Means (K)**
	A0	**A1**	**A2**		**A0**	**A1**	**A2**	
K0	43.00	44.48	44.56	44.34	7.10	7.39	7.48	7.32

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K1	43.85	44.90	45.73	44.83	7.36	7.45	7.75	7.52
K2	44.63	45.35	45.46	45.14	7.27	7.84	8.02	7.71
K3	45.67	45.40	46.00	45.69	7.54	7.83	8.30	7.89
Means (A)	44.29	45.03	45.68	**Means (A)**	7.31	7.63	7.88	
LSD 0.05	**K**	**A**	**KXA**		**K**	**A**	**KXA**	
	0.46	0.40	0.80		0.11	0.10	0.20	

The reason for increase in the percentage of dry matter in the leaves as well as the shoots content of carbohydrates may be due to the strength and activity of vegetative growth of the young trees resulting from spraying with kinetin and (Vegeamino) which is represented by increasing the number and length of branches and the number and area of leaves as shown in Tables (3 and 4), in addition to its content of chlorophyll and its reflection in increasing the efficiency of photosynthesis and an increase the carbohydrates synthesis (Jordan and Ogren, 1984), these results agree with Abou Aziz et al. (2011) which founds that spraying olive trees with benzyl adenine has increased the dry weight of leaves and their carbohydrates content, it also agree with the results of El-Badawy and Abd El-Aal (2013) where the dry weight of the leaves and their carbohydrates content increased significantly for the mango saplings when spraying with the kinetin, it also agreed with El-Shazly and Mustafa (2013), who reported a significant increase in the dry weight of the leaves when sprayed with a mixture of amino acids (Amino green II) on orange trees, also agree with Ali et al. (2019), where they found that spraying by amino nutrient has led significantly to increase the shoots content of total carbohydrates of olive trees.

4- Leaves content of nitrogen (%) and their content of total chlorophyll (mg. 100gm^{-1} fresh weight):

The study treatments affected the leaves content of nitrogen as well as its content of total chlorophyll. Table (6) shows that the spraying with kinetin at the concentration of K2 achieved a significant increase by giving it the highest values which amounted to 1.69% and 1.23 mg.100g^{-1} fresh weight compared to the K0 treatment which recorded the lowest values reached 1.62% and 118.33 mg.100g^{-1} fresh weight**.** The foliar spraying with amino acids showed a significant increase in these two traits, especially the A2 level which achieved the highest values amounted to 1.68% and 123.25 mg.100g^{-1} fresh weight compared to the A0 level which recorded the lowest values amounted to 1.64% and 119.33 mg.100g^{-1} fresh weight. The interaction between the two factors showed its significant effect by achieving the treatment K3 X A2 the highest leaves content of nitrogen and total chlorophyll, which reached 1.71% and 126.33 mg.100g^{-1}fresh weight, while the K0 X A0 treatment achieved the lowest values amounted to 1.61% and 117.33 mg.100g^{-1} fresh weight.

Table 6: Effect of foliar application with kinetin and amino acids and their interaction in the leaves content of nitrogen and their total chlorophyll of young olive trees

Leaves content of nitrogen (%)				**Leaves content of total chlorophyll (mg.100g^{-1} fresh weight)**				
Kinetin (K) mg.L^{-1}	**Amino acids (A) ml.L^{-1}**			**Means (K)**	**Amino acids (A) ml.L^{-1}**			**Means (K)**
	A0	**A1**	**A2**		**A0**	**A1**	**A2**	
K0	1.61	1.62	1.64	1.62	117.33	118.33	119.33	118.33
K1	1.67	1.64	1.71	1.67	122.67	122.33	123.67	122.89

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K2	1.62	1.71	1.67	1.66	118.00	120.67	123.67	120.78
K3	1.65	1.71	1.71	1.69	119.33	123.33	126.33	123.00
Means (A)	1.64	1.67	1.68	**Means (A)**	119.33	121.17	123.25	
LSD 0.05	**K**	**A**	**KXA**		**K**	**A**	**KXA**	
	0.02	0.02	0.04		0.65	0.56	1.13	

The reason for increase the concentration of nitrogen in the leaves as a result of treatment with kinetin to its stimulating role in the uptake and transport of nutrient elements in the plant towards the treated tissues with it (Mok, 1994; Beck, 1996). Also, the increase in the percentage of nitrogen as a result of spraying with amino acids may be due to its important role which represented the source for organic nitrogen (Endres and Mercier, 2003; Tegeder, 2012), which absorbed directly when sprayed on the leaves, also can be attributed to the effect of the study factors in increasing the vegetative growth of the young trees (Table 3 , 4), which positively affects their ability to absorb the relatively available mineral elements in the soil as shown in (Table 1) to achieve nutritional balance in plant.

The reason for the increased the leaves content of total chlorophyll as a result of spraying with kinetin, it may be due to its role in stimulating the biosynthesis of chlorophyll by activating the enzyme NADH-Protochorophyll, in addition to its role in stimulating chloroplast development (Zavaleta-Mancera et al., 1999). The reason for the increased chlorophyll concentration as a result of treatment with amino acids may be due to its role in biosynthesis that pigment especially Glycine and Glutamic acid (D'Mello, 2015). Also maybe due to its effect of the study factors in increasing the leaves content of nitrogen as shown in table (6), which is involved in the construction of the basic Porphyrin ring in the structure of the chlorophyll molecule, where 75% of the nitrogen presence in mesophyll cells, Its location is chloroplasts (Peoples and Dallin, 1988; Stefan and Feller, 2001). These results correspond with Mahmoud et al. (2015) where the leaves content of olive trees was significantly increased from nitrogen and chlorophyll when spraying it with benzyl adenine, it also agreed with the results of Al-Janabi (2018) when spraying the cleopatra mandarin saplings with kinetin, which significantly increased the concentration of nitrogen and total chlorophyll in leaves, also consistent with the results of Abd El-Razek and Saleh (2012) which showed that the content of leaves peach (*Prunus persica*) trees from N and chlorophyll were significantly increased when spraying it with amino nutrient of Pepton$^{85/16}$, it also agrees with Al-Janabi (2020) who obtained a significant increase in the concentration of nitrogen and chlorophyll in the leaves of sweet orange saplings when spraying it with amino acids solution.

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