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Effect of Foliar Application of Free Amino Acids on Salt Tolerance and Yield Quality of Manzanillo Olive Tree

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Abstract: This study aimed to investigate the impact of different types of free amino acids (proline, glutamic acid, and GABA), applied either individually or in combination, on the vegetative growth and yield of nine-year-old Manzanillo olive trees over two consecutive seasons (2021 and 2022) in a private orchard located along the Cairo-Alexandria desert road, Egypt (approximately 64 km from Cairo). The findings revealed that applying a mixture of free amino acids at a rate of 50 ppm positively influenced vegetative growth, flowering, fruit physical properties, and yield, with no significant differences compared to foliar application of 50 ppm glutamic acid alone. Additionally, the combined amino acid treatment enhanced the olive trees' resistance to salt stress by increasing total amino acid content in the leaves while reducing carbohydrate content.

Key words: Olive, amino, proline, glutamic, gaba, productivity.

1. Introduction

The olive tree (*Olea europaea* L.) is among the most economically significant fruit crops globally, valued for its oil and table olives. However, its cultivation faces numerous challenges, particularly abiotic stresses, with salinity being a major concern, especially in arid and semi-arid regions. Salt stress adversely affects plant physiology by causing osmotic imbalance, oxidative damage, and ion toxicity, ultimately resulting in diminished growth, yield, and quality. **(Lodolini *et al.*, 2019; Al-Maaitah *et al.*, 2017 and Nogueira *et al.*, 2022).** Amino acids, as crucial components of plant metabolism, have garnered attention for their potential to ameliorate salt stress effects in olive trees.

The purpose of this review is to present a comprehensive understanding of the uses of amino acids to improve salt tolerance in olive cultivation and to identify future research directions for optimizing their application in practical agricultural settings. The use of foliar-applied amino acids to mitigate these adverse effects, enhancing the resilience and productivity of olive trees in saline environments. This review summarizes recent research findings on the role of amino acids in improving salt tolerance and yield quality in olive cultivation **(Zrig *et al.*, 2023 and Di Vaio *et al.*, 2024).**

Recent research by **Zhang and Zhou (2020) and Kumar and Singh (2020**) has highlighted the roles of amino acids in maintaining ion homeostasis and synthesizing stress-responsive metabolites, respectively. Studies by **Ben Ahmed *et al.* (2020) and Zrig *et al.* (2023)** have provided valuable insights into how amino acids influence hormonal signaling and metabolic adjustment in olive trees subjected to saline environments. Amino acids serve as precursors for the synthesis of stress-responsive metabolites and osmoprotectants in olive trees. They participate in the biosynthesis of compatible solutes, polyamines, and secondary metabolites that have a role in the mechanisms of stress tolerance (**El-Tayeb, 2021; Liu *et al.*, 2022 and El-Esawi and Ali 2022).**

Amino acids such as proline and glycine betaine have been shown to accumulate in plants under salinity stress. These compounds act as osmoprotectants, helping cells maintain turgor pressure and preventing ion imbalance by stabilizing cellular structures. Recent studies highlight that proline application leads to a significant reduction in oxidative damage by modulating osmotic adjustment and ion transport **(Ghars *et al.*, 2022).**

Amino acids contribute to osmotic adjustment by accumulating in plant cells, thereby maintaining turgor pressure and water uptake under saline conditions. Proline, glycine betaine, and arginine are among the most prominent amino acids implicated in osmotic regulation in olive trees subjected to salt stress **(Iqbal *et al.*, 2021; Farooq *et al.*, 2020; Orlandi *et al.*, 2021 and Hassan and Ali 2022).**

Salinity stress triggers the production of reactive oxygen species (ROS), causing oxidative damage in plants. Amino acids such as cysteine and methionine serve as precursors for key antioxidants like glutathione. Applying these amino acids as foliar sprays enhances the activity of antioxidant enzymes, including superoxide dismutase (SOD) and peroxidase (POD), thereby mitigating oxidative stress and enhancing the plant's tolerance to salinity. **(Hasanuzzaman *et al.*, 2020; Ahmad and Sharma 2021; El-Esawi *et al.*, 2020; Zrig *et al.*, 2023 and Mosleh & Soltani, 2023).**

One key aspect of salinity stress is the disruption of ion balance within plant tissues. Exogenous application of amino acids enhances the selective absorption of essential ions like potassium (K^+) while limiting the accumulation of toxic ions like sodium (Na^+). Excessive sodium uptake under salt stress disrupts ion homeostasis in olive plants. Amino acids play a role in ion compartmentalization and sequestration, thereby minimizing the toxic effects of sodium and maintaining ion balance within cells **(Ali *et al.*, 2021 and Hamed *et al.*, 2021).** Recent research in 2023 revealed that foliar application of amino acid blends markedly enhances nutrient uptake efficiency, promoting improved growth in plants under saline conditions. **(Zrig *et al.*, 2023 and Di Vaio *et al.*, 2024).** Cysteine, glutamine, and asparagine are implicated in ion homeostasis mechanisms in olive trees exposed to saline environments **(Hu *et al.*, 2015; Zhang and Zhou 2020 and Wang *et al.*, 2022).**

Amino acids like tryptophan are precursors for plant hormones such as auxins. Studies show that foliar application of tryptophan increases auxin levels, promoting root development and improving water and nutrient uptake. This hormonal modulation helps maintain vegetative growth and yield even in saline environments **(Mosleh and Soltani, 2023).** Amino acids influence hormonal signaling pathways involved in plant stress responses. Amino acid blends regulate the biosynthesis, perception, and signaling of stress-related hormones, including abscisic acid (ABA), cytokinins, and jasmonates, in olive trees. This modulation helps coordinate adaptive responses to salt stress **(Zhang and Jiang 2021; Fariduddin *et al.*, 2021; Khan and Müller 2022).**

Recent studies from 2022 and 2023 emphasize the beneficial effects of amino acid foliar sprays on olive fruit yield and quality under salinity stress. These findings indicate that such applications enhance oleic acid content and improve oil stability, both of which are essential quality attributes for olive oil **(Ghars *et al.*, 2022).** Salinity stress often leads to poor fruit set and reduced yields. Amino acid treatments, particularly those including proline, arginine, and glutamine, have been linked to increased fruit retention, improved chlorophyll content, and higher overall yields **(Zrig *et al.*, 2023).** Amino acid treatments contribute to better nutrient translocation and cell enlargement, resulting in improved fruit size and weight even under stress conditions **(Di Vaio *et al.*, 2024).**

This study aimed to investigate the impact of individual free amino acids and a mixture of three amino acids on salt tolerance and yield performance of Manzanillo olive trees.

2. Materials and Methods

A field study was conducted over two consecutive growing seasons (2021 and 2022) on 25 nine-year-old Manzanillo olive trees grown in sandy soil under a drip irrigation system. The trees, spaced 4 x 6 meters apart, were located in a private orchard 64 kilometers from Cairo, at coordinates 30°268'215" N latitude and 30°806'534" E longitude. The study aimed to investigate the role of amino acids in enhancing olive tree growth and yield. All selected trees were disease-free, uniform in size and shape, and irrigated with groundwater. Standard cultural practices and fertilization were applied. Soil physical and chemical characteristics, along with water chemical properties, were analyzed by the Soil, Water, and Environmental Research Institute of the Agricultural Research Centre, using the methods described by **Wilde *et al.* (1985).** The results of these analyses are summarized in Tables 1 and 2.

Table (1). Physical and chemical analysis of the experimental soil

Physical analyses (%)									
Coarse sand			Fine sand		Silt		Clay	Texture class	
38.4			43.3		13.0		5.3	Sandy	
Chemical analyses (Anions and Cations) mg/L									
pH	ECds /m	Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO^{--}	HCO_3^{-}	Cl^{-}	SO_4^{--}
7.99	0.56	1.8	0.87	2.5	1.25	--	1.51	4.51	0.43
Available nutrients (mq/ Kg Soil)									
N		P		K					
127.3		15.8		99.8					

Table (2). Chemical characteristics of the tested water sample collected from the experimental area

		Soluble cations(me/L)				Soluble anions (me/ L)				
pH	E.C. ds/M (1:5)	Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO^{--}	HCO_3^{-}	Cl^{-}	SO_4^{--}	SAR
7.12	6.10	22.69	16.54	16.54	0.17	-	1.52	18.75	47.91	6.25

The study was designed in a complete randomized block design with three replicates, two trees were used as a separator between treatments. The selected trees received the following foliar spray treatments during swelling buds stage and before the beginning of flowering:

1- Control (sprayed with water only).

2- Glutamic amino acid at 50 ppm.

3- Proline amino acid at 50 ppm.

4- GABA amino acid at 50 ppm.

5- A mixture of previous amino acids at 50 ppm.

The Following Parameters were estimated:

2.1. Vegetative Growth Measurements

Measurements such as shoots length (cm), No. of leaves/shoot and Leaf area (cm^2) were made in August of each season on sixteen healthy shoots (4 in each tree direction) which selected randomly and labeled. Leaf area (cm^2) were measured by using the planimeter.

2.2. Flowering Behavior

The following behaviors were estimated and recorded: No. of inflorescence/shoot, No. of flower/ inflorescence, sample of inflorescences were randomly taken at bloom stage from the middle portion of shoot from each replicate and No. of flower were counted. Sex ratio % it was calculated (Sex ratio % = perfect flower/ total No. of flower * 100).

2.3. Fruit physical characteristics

Physical characteristics of olive fruits such as Fruit shape index (fruit length/ fruit diameter), seed shape index (seed length/ seed diameter) and Pulp % (pulp weight/ fruit weight * 100) were determined at harvesting time.

2.4. Yield

The number of fruits per shoot, fruits per meter, and the average yield per tree (kg/tree) were recorded at harvest time (October) for each treatment during both growing seasons. Fruit weight was measured using an electric balance, with samples consisting of 10 fruits per replicate. The average fruit weight was then calculated. The oil content (%) of dried fruit samples was determined using petroleum ether (60–80%) as a solvent over 16 hours with a Soxhlet apparatus, following the procedure outlined by **A.O.A.C. (1998).**

2.5. Chemical Analysis

- Total amino acids

The total amino acids in olive leaves was determined according to **Yemm and Cocking (1995).**

- Total carbohydrates

Total sugars (carbohydrates) were determined according to the method of **Dubois *et al.* (1956).**

2.6. Data Analysis

The obtained data were statistically analyzed **(Senedecor and Cochran, 1990)**. Duncan s multiple range tests effect was used to compare treatment means **(Duncan, 1955)**.

3. Results and discussion

3.1. Vegetative growth

Data in Table (3) showed a significant difference among different treatments during two studied seasons. the highest significant shoot length (cm) were recorded by all amino acid treatments. While the least shoot length (17.29 – 17.33 cm) came from the control treatments in both seasons respectively.

Regarding the number of leaves per shoot, data presented in Table (3) indicate that amino acid treatments did not produce any significant effect during the first season. However, in the second season, the highest number of leaves per shoot was observed in the control treatment, recording 53.42 leaves.

Also, data indicate that leaf area of Manzanillo olive cultivar were ranged from (3.29 – 4.60 cm^2) in the 1st season and from (3.55- 4.94 cm^2) in the second one. The highest leaf area was recorded by the foliar spray with a mixture of amino acids in the 1st and 2nd seasons, respectively.

The positive effect of amino acids on vegetative growth of olive tree may explained by its function on protein synthesis and the formation of enzymes important for the metabolism process **(Aberg, 1961 and Mervat *et al.*, 2015)**. Additionally, **Davies (1982)** and **Feng *et al.* (2020)** stated that amino acids help regulate cellular pH due to their buffering capacity and metabolic roles. This regulation is critical for maintaining an environment conducive to enzymatic reactions and cellular metabolism. Moreover, in case the plant faces a deficiency of carbohydrates, Amino acids give plant cells an instant source of nitrogen and can also serve as a source of carbon and energy (**Goss, 1973: Thon *et al.*, 1981, Calvo *et al.*, 2014** and **Zayed *et al.*, 2023).**

Table (3). Effect of foliar spray with amino acids on vegetative growth of olive trees cv. Manzanillo on two studied seasons (2021 and 2022)

Treatment	Shoot length (cm)		No. of leaves		Leaf area (cm^2)	
	1 st	2 nd	1 st	2 nd	1 st	2 nd
Control	17.29 B	17.33 C	38.42 A	53.42 A	3.90 B	4.00 C
Glutamic	20.48 A	20.25 ABC	37.08 A	43.92 B	4.04 B	4.24 B
Proline	18.64 AB	18.12 BC	43.67 A	45.50 B	3.29 C	3.55 D
GABA	19.58 AB	22.04 A	38.92 A	40.33 B	4.14 B	4.16 BC
Mix	19.97 AB	20.75 AB	37.58 A	45.00 B	4.60 A	4.94 A

Values sharing the same letter are not significantly different at the 5% significance level.

The obtained results are in harmony with those stated by **Psarras *et al.* (2024)** on olive and **El-Badawy (2019)** on apricot**. Nafea and Al-Janabi (2021)** illustrated that olive shoot length increased significantly by the using of amino acid. In addition, **El-Bolok and Kasem (2023)** observed an enhancement on the vegetative growth of olive trees c.v Agazzi when it sprayed with chitosan at 1% + amino acids at 2%.

3.2. Flowering Behavior

The data in Table (4) reveal that olive flowering behavior was gradually increased by using amino acids. As such, the foliar spray with Glutamic amino acid gave the highest significant No. of inflorescences/ shoot, No. of flower/inflorescence and sex ratio % in two studied seasons without significant differences with those treated by the mixture of tested amino acids in most cases.

Amino acids have the capacity to regulate plant stress responses and this reflected in improving floral behavior. They have the ability to regulate the levels of important phytohormones like salicylic acid and abscisic acid (ABA), which are involved in plant development, stress resistance, and flowering**.** In addition, **Psarras *et al.* (2024)** illustrated that, No. of inflorescences of olive ‘Koroneiki cv.’ were increased significantly when it treated by amino acid spray.

Table (4). Effect of foliar spray with amino acids on flowering behavior of olive trees cv. Manzanillo on two studied seasons (2021 and 2022)

Treatment	No of inflorescence		No. of flower		Sex ratio	
	1 st	2 nd	1 st	2 nd	1 st	2 nd
Control	6.25 B	11.83B	11.83B	15.00A	47.09AB	66.50C
Glutamic	9.75A	14.33A	13.67 A	15.33A	58.11A	86.39AB
Proline	8.67 AB	7.58 C	12.58AB	14.33A	37.45B	74.62C
GABA	7.58 AB	9.25 C	12.92AB	14.17A	51.45AB	83.71B
Mix	10.00A	12.00 B	13.67 A	15.00A	43.52B	93.98A

Values sharing the same letter are not significantly different at the 5% significance level.

3.3. Fruiting and fruit character

No. of fruit/shoot

The results in Table (5) indicate that the application of amino acids significantly increased the No. of fruits per shoot in treated plants compared to the untreated control. In the 1st season, the highest No. of fruits per shoot was recorded with the spraying of Glutamic amino acid, resulting in 5.92 fruits per shoot. In the 2nd season, a mixture of amino acids, including Glutamic acid, Proline, and Gapa, resulted in the highest No. of fruits per shoot at 7.67. These findings suggest that amino acid treatments, particularly Glutamic and the amino acid mix, positively influenced fruit production across both seasons. In the same order of amino acid treatments, regardless of the variance between them, the lowest mean No. of fruits per shoot was observed under Proline treatment, which recorded 3.08 fruits per shoot in the 1st season. Meanwhile, in the 2nd season, the control (untreated) treatment showed the lowest value, with 4 fruits per shoot. This further emphasizes that while amino acid treatments generally increased fruit production, Proline was the least effective in the 1st season, and untreated plants had the lowest yield in the 2nd season.

The effectiveness of the Glutamic acid in enhancing fruit production aligns with research indicating glutamic acid plays a significant role in plant growth and development. Glutamic acid has been shown to improve various plant physiological processes, which can enhance fruit characteristics **(Eisaa *et al.*, 2023).** Similarly, the amino acid mix's superior performance in the 2nd season supports findings that combining amino acids can synergistically benefit plant growth and fruit yield **(Liu *et al.*, 2020).**

In contrast, Proline treatment yielded the lowest No. of fruits per shoot in the 1st season (3.08 fruits), which suggests that Proline alone may be less effective in promoting fruit production compared to other amino acids or combinations. The control treatment also showed the lowest fruit production in the 2nd season (4 fruits per shoot), highlighting the overall benefits of amino acid treatments. This observation is consistent with studies that emphasize the advantages of amino acid supplementation over no treatment **(Ekşi and Sönmez, 2022).**

Number of fruits/meter

The results presented in (Table 5) illustrated that the application of various amino acids significantly affected the mean No. of fruits per meter. Spraying with amino acids resulted in a substantial increase in the No. of fruits per meter compared to the control. There was significant variance among the treatments with Glutamic, Proline, Gapa, and the amino acid mix across both seasons. The highest values were recorded with the Glutamic treatment, achieving 31.80 fruits per meter in the 1st season and 36.55 in the 2nd season. Additionally, the amino acid mix (Glutamic, Proline, and Gapa) recorded the highest value in the 2nd season at 38.75 fruits per meter. These findings highlight the positive impact of Glutamic and the amino acid mix on fruit yield. The lowest mean values of fruits per meter were observed with Proline (16.61) in the 1st season and in the control treatment (23.5) during the 2nd season. This suggests that while amino acid treatments generally enhanced fruit yield per meter, Proline was the least effective in the 1st season, and untreated plants had the lowest fruit yield in the 2nd season.

This result supports the role of Glutamic in promoting fruit production. Glutamic acid improves plant tolerance by enhancing water retention, reducing oxidative stress, and increasing antioxidant enzyme activity. Also, it boosts proline levels for osmotic balance and ABA accumulation, activating drought-resistance pathways that strengthen cellular defenses and reduce damage under stress, **(Xu, *et al.*, 2020)** these actions can lead to increased fruit yield. Furthermore, the amino acid mix, which includes Glutamic acid, Proline, and Gapa, achieved the highest fruit yield in the 2nd season (38.75 fruits per meter). This finding is consistent with studies suggesting that combinations of amino acids can have synergistic effects on plant growth and fruit production **(Liu *et al.*, 2020).**

The significant variance among treatments indicates that not all amino acids had the same impact on fruit yield. Proline treatment yielded the lowest No. of fruits per meter in the 1st season (16.61 fruits), and the control treatment recorded the lowest fruit yield in the 2nd season (23.5 fruits). Proline, while beneficial in some contexts, was less effective compared to glutamic and the amino acid mix, which might be due to its limited role in enhancing overall fruit production under the conditions tested. The control's lower fruit yield further underscores the advantages of amino acid supplementation over no treatment.

Fruit shape index

Table (5) shows that the application of all amino acid treatments led to an increase in the fruit shape index compared to the untreated plants, which had the lowest values (1.11 and 1.16 as the mean for both seasons). The greatest fruit shape index was observed under the glutamic amino acid treatment, with mean values of 1.30 in the 1st season and 1.36 in the 2nd season. This indicates that glutamic amino acid treatment had the most significant effect on improving the fruit shape index across both seasons.

The significant improvement in fruit shape index with glutamic amino acid treatment aligns with existing literature that highlights the role of amino acids in fruit development. glutamic acid is known to influence various plant physiological processes, including growth and stress responses. Its application can enhance cellular metabolism and improve overall fruit quality, including shape and uniformity **(Brosnan and Brosnan, 2013; Almutairi *et al.*, 2022).** The superior performance of glutamic in improving the fruit shape index could be attributed to its role in supporting root growth, calcium transport, and hormonal balance.

Seed shape index

The results presented in Table (5) indicate a significant effect of amino acid treatments on the seed shape index in both seasons. The foliar spray with glutamic amino acid produced the highest seed shape index values, with 1.83 in the 1st season and 1.92 in the 2nd season. The differences among the other treatments were insignificant, further highlighting the superior influence of glutamic amino acid on seed shape index compared to the other amino acids.

Pulp %

Table (5) illustrated the pulp percentage (%), showing that the mix of amino acids was the most effective treatment, resulting in 87.18% and 86.26% pulp in the 1st and 2nd seasons, respectively. The lowest pulp percentage was recorded in the control, Glutamic, and Gapa amino acid treatments during the 1st season. While there were no significant differences between most treatments, the amino acid mix demonstrated a notably superior effect on pulp percentage across both seasons.

This finding aligns with previous research suggesting that amino acid treatments can enhance various fruit characteristics, including pulp quality. For instance, studies have shown that mixtures of amino acids can synergistically enhance plant growth and fruit development by optimizing nutrient uptake and physiological processes **(Ekşi and Sönmez, 2022).** This observation suggests that while individual amino acids can improve certain fruit characteristics, their effects on pulp percentage might not be as pronounced as those achieved with a combination of amino acids. Glutamic acid and Gapa have been reported to affect plant metabolism and stress responses **(Zhang and Jiang, 2021),** but their impact on pulp percentage might be limited compared to a well-balanced amino acid mixture. It is possible that the effects of individual amino acids or specific combinations may vary depending on the plant species, growth conditions, and fruiting stage**.**

Table (5). The effect of amino acids on fruiting and fruit characteristics of olive trees cv. Manzanillo on two studied seasons (2021 and 2022)

Treatment	No. of fruit/shoot		No. of fruit/meter		Fruit shape index		Seed shape index		Pulp %	
	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd
Control	3.83^{B}	4.00^{D}	22.73^{AB}	23.50^{B}	1.11^{C}	1.16^{C}	1.78^{AB}	1.76^{C}	84.28^{C}	84.20^{B}
Glutamic	5.92^{A}	7.25^{AB}	31.80^{A}	36.55^{A}	1.30^{A}	1.31^{AB}	1.83^{A}	1.82^{B}	83.67^{C}	83.68^{B}
Proline	3.08^{B}	4.75^{CD}	16.61^{B}	27.49^{B}	1.26^{AB}	1.36^{A}	1.76^{B}	1.85^{B}	85.53^{B}	86.26^{A}
Gapa	4.67^{AB}	6.08^{BC}	23.37^{AB}	31.54^{AB}	1.25^{AB}	1.34^{AB}	1.75^{B}	1.84^{B}	84.02^{C}	84.57^{B}
Mix	4.08^{B}	7.67^{A}	19.89^{B}	38.75^{A}	1.22^{B}	1.27^{B}	1.77^{B}	1.92^{A}	87.18^{A}	84.38^{B}

Values sharing the same letter are not significantly different at the 5% significance level.

Fruiting and fruit character

Yield kg/tree

The data in Table (6) show that during the first experimental season, a glutamic spray at 50 mg/L significantly increased the yield to 18.3 kg/tree. In the 2nd season, a mix of amino acid treatments led to a yield increase of 33.5 kg/tree. However, there were no significant differences between the control, proline, and GAPA treatments, all of which resulted in the lowest yields per tree in the 1st season. In contrast, during the 2nd season, the control treatment produced the lowest yield at 13.87 kg/tree.

This suggests that glutamic acid plays a key role in improving nutrient absorption and overall plant metabolism, leading to enhanced growth and productivity **(Shehata *et al.*, 2021).** Glutamic acid is known to act as a precursor for other amino acids and has been shown to improve the efficiency of nitrogen use in plants, which likely contributed to the yield increase observed in this season **(Khalil & Hussein, 2020).**

Fruit weight (10 fruits)

From the results shown in Table 6, it is evident that the spray mix of amino acids had a significant effect. Notably, the 50 mg/L level produced the highest fruit weights, recording 47.07 g/10 fruits and 46.37 g/10 fruits in the 1st and 2nd seasons, respectively. In contrast, the lowest fruit weights were observed with the GAPA amino acid treatment, which resulted in 37.98 g/10 fruits and 41.17 g/10 fruits in the 1st and 2nd seasons, respectively.

These results demonstrate the positive role of amino acids in promoting fruit development, likely due to their involvement in various physiological processes, such as nutrient absorption, protein synthesis, and enzyme activation **(Shehata *et al.*, 2021).** Amino acids are known to improve overall plant vigor, which can directly contribute to increased fruit size and quality.

Oil %

The same trend in Table 6 also shows that the application of the amino acid mix at 50 mg/L had a significant positive effect, outperforming the control treatment. This treatment resulted in the highest oil content, with 23.7% in the 1st season and 25.8% in the 2nd season. On the other hand, the control treatment produced the lowest oil content in the fruits, with 21.60% in the 1st season and 22.33% in the 2nd season.

These findings align with previous research indicating the critical role of amino acids in plant growth, fruit development, and oil biosynthesis. Amino acids, as the building blocks of proteins, play an essential role in plant metabolism. They participate in the synthesis of enzymes and hormones that regulate various physiological processes, including nutrient uptake, stress tolerance, and secondary metabolite production **(Shehata *et al.*, 2021).** Specifically, amino acids such as proline, glutamic acid, and others can enhance the plant's capacity to assimilate nutrients and produce metabolites, which are crucial for oil synthesis in olive trees **(Khalil & Hussein 2020).** Amino acids promote the accumulation of oil in fruits by boosting the activity of enzymes involved in lipid biosynthesis. Additionally, these amino acids are known to enhance chlorophyll content, photosynthetic efficiency, and carbon fixation, all of which contribute to improved fruit quality and increased oil content (**Abdel-Mawgoud *et al.*, 2020).**

Table (6). The effect of amino acids on yield (Kg/ tree), fruit weight (g/10 fruits) and oil % of olive trees cv. Manzanillo on two studied seasons (2021 and 2022)

Treatment	Yield		Fruit weight (10 fruit)		Oil %	
	1 st	2 nd	1 st	2 nd	1 st	2 nd
Control	6.22 C	13.87C	40.37BC	42.80BC	21.60 D	22.33D
Glutamic	18.30A	23.17B	41.26B	44.73AB	23.47AB	24.67B
Proline	7.50 C	15.50C	39.87BC	42.73BC	22.86BC	23.40C
Gapa	7.63 C	22.33B	37.98 C	41.17C	22.40CD	22.83CD
Mix	12.18B	33.50A	47.07A	46.37A	23.70A	25.80A

Values sharing the same letter are not significantly different at the 5% significance level.

Chemical contents in olive leaves

Total amino acid content (g/100 g)

The results for amino acid content presented in Table (7) show significant variation among the different treatments. The application of the amino acid mixture resulted in a notable increase in total amino acid content during both study seasons, ranging from 0.0625 to 0.0580. The control treatment, however, exhibited the lowest amino acid content values, while the other treatments yielded intermediate values in both seasons.

Total carbohydrate (%)

Data in Table (7) showed the effect of foliar application of different types of amino acids on carbohydrate (%) content in olive leaves. the control treatment recorded statistically the highest carbohydrate content (33.00- 34.50 %) in the two investigated seasons. Meanwhile, the lowest carbohydrate content was achieved by the mixture of amino acid treatment (17.22- 15.71 %) in the 1st and 2nd seasons, respectively.

The accumulation of carbohydrate seems to be one of the survival mechanism for plants grown under salt stress (**El Yamani and Cordovilla, 2024)**. In abiotic reactions, carbohydrates serve as substrates for osmotic regulation, protection, and energy generation that connect growth development and carbon status (**Diniz *et al*., 2020)**. In addition, **Tattini *et al.*,** (2006) showed that, carbohydrates increased as the external NaCl concentration was increased.

Table (7) the effect of amino acids on total carbohydrate and amino acid content in olive leaves.

Treatment	Total amino acid g/100 g		Total carbohydrate %	
	1 st	2 nd	1 st	2 nd
Control	0.0400 d	0.0435 c	33.00 a	34.50 a
Glutamic	0.0485 b	0.0500 b	19.82 d	19.92 c
Proline	0.0405 d	0.0455c	28.54 b	25.92 b
Gapa	0.0425 c	0.0460 c	22.02 c	20.22 c
Mix	0.0625 a	0.0580 a	17.22 e	15.71 d

Values sharing the same letter are not significantly different at the 5% significance level.

4. Conclusion

Based on obtained data in this research, it could be noted that the foliar application of Glutamic amino acid alone significantly was the best treatment compared to other free amino acid during this study and mixture of free amino acid enhance the vegetative growth and productivity of olive trees (Manzanillo cv.).

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