Journal of Applied Horticulture, 28(1): 200-205, 2026

https://doi.org/10.37855/jah.2026.v28i01.31

Journal of Applied Horticulture
ISSN: 0972-1045

Foliar application of supplemental IBA combined with ascorbic acid or quercetin to stimulate rooting in Kalamata and Wardan olive cuttings

Ahmed Abdelhady Rashedy*[1], Omar Thaer Kamil[2], Ahmed Adnan Mohammed[2], Wael Mohamed Eldeeb[3] and Hamed Hosny Hamed[1,4]

*[1]Pomology Department, Faculty of Agriculture, Cairo University, Giza, Egypt. [2]Department of Medicinal and Industrial Plants, College of Medicinal and Industrial Plants, University of Kirkuk, Kirkuk, Iraq. [3]Energy and Environment Research Center, Tabbin Institute for Metallurgical Studies, Egypt. [4]Organic Crop Production Department, Faculty of Organic Agriculture, Heliopolis University, Egypt. *E-mail: ahmed.rasheedy@agr.cu.edu.eg*

Abstract

Indole-3-butyric acid (IBA) is a primary growth regulators that promote rooting. Quercetin (QC) is a flavonoid antioxidant that has recently been recognized as a co-factor in the rooting of Kalamata olive cuttings. Also, Ascorbic acid (ASC) successfully stimulates rooting in some olive cuttings. The objective of this study was to evaluate the efficacy of foliar application of IBA (0, 1000 ppm) after cuttings planting, combined with either ASC (50, 100 ppm) or QC (50, 100 ppm), on rooting of Kalamata and Wardan olive cuttings. The results showed that the effect of individual IBA on promoting rooting in Kalamata cuttings was minimal, whereas the combined application of IBA+ASC was more effective than IBA+QC. In Wardan cuttings, individual IBA had a negative effect on rooting, while the combined application of IBA+ASC significantly improved rooting %, followed by IBA+QC. In both cultivars, promoting rooting was associated with increased activity of peroxidase (POD) and catalase (CAT), while activity of polyphenol oxidase (PPO) and indole-3-acetic acid oxidase (IAAox) decreased. ASC alone recorded the highest rooting percentage at 12.1% in Kalamata cuttings compared to the control (0%), and 54.4% in Wardan cuttings compared to the control (14.9%). The highest rooting % was accompanied by decreases in IAAox and PPO of 13.33% and 30.16% for Kalamata cuttings and 17.27% and 27.21% for Wardan cuttings. In comparison, it coincided with increases of 28.74% and 497.4% in CAT and POD in Kalamata, and 31.21% and 641% in the Wardan cultivar.

Key words: *Olea europaea* L., CAT, POD, PPO, IAAox

Introduction

Olive (*Olea europaea* L.) is one of the most common and oldest fruit trees belonging to the Oleaceae family and originated in the Mediterranean basin; it later spread to Asia, South America, and Australia (Afridi *et al*., 2015). Also, it is more suitable for arid regions due to its specialized leaf and ramified root structures (Tanasijevica *et al*., 2014). Egypt is considered one of the top-producing countries, accounting for about 23% of global production (520,000 tons in 2023/2024) and ranking as the third-largest exporter outside the EU (IOC, 2025).

Leafy olive, also known as semi-hardwood cuttings, is considered the primary propagation method for olive (Mohammed, 2021; Rashedy *et al*., 2021; Kamil *et al*., 2025). Unfortunately, there is variation in the rooting ability of olive cultivars, with easy-to-root cultivars like Coratina (Rashedy *et al*., 2021) and hard-to-root cultivars like Kalamata (El-Sayed *et al*., 1995; Denaxa *et al*., 2020), and intermediate cultivars like the Wardan cultivar (Gowda *et al*., 2010). Also, cuttings propagation is affected by many factors, such as temperature, relative humidity, and the time of propagation, which are correlated with plant growth-promoting or inhibitory content. In Egypt, like many Mediterranean countries, during the winter season the temperature drops below 10°C. Also, the high cost of electricity and the need for fog irrigation systems make the production of olive cuttings under a plastic tunnel more suitable (Rashedy *et al*., 2021).

Auxin, especially indole-3-butyric acid (IBA) is considered the most important plant growth regulator stimulate rooting of many cuttings such as olive (Kamil *et al*., 2025; Amin and Baqi, 2025), Guava (El-Sharony *et al*., 2018), grapes (Asma *et al*., 2025; Othman and Hawezy, 2022), vegetables (Hoque *et al*., 2025; Samad *et al*., 2022) and tissue culture (Rehman *et al*., 2025; Mir *et al*., 2025). Furthermore, exogenous IBA increases endogenous IAA levels and decreases ABA levels (Khana *et al*., 2024).

ASC is considered an anti-oxidative substance (Patel *et al*., 2020), which is commonly used in tissue culture and is used as a rooting stimulator in many plants such as guava (El-Sharony *et al*., 2018), *Stewartia pseudocamellia* (Struve and Lagrimini, 1999) and *Acacia leprosa* (Radhi and Hussein, 2020). ASC regulates ROS scavenging (Hasanuzzaman *et al*., 2020), cell multiplication (Ellya Kka, 2017), cell elongation (Paciolla *et al*., 2019), and enzyme activity (Li *et al*., 2007).

Quercetin (QC) is one of the phenolic compounds that increase cell division and alleviate oxidative stress (Tahoori *et al*., 2019), preventing the oxidation of indole-3-acetic acid and enhancing its movement, ultimately bolstering the rooting ability of cuttings (Englert *et al*., 1991) of many plants (Alharbi *et al*., 2025)and Malus Jork 9 (De Klerk *et al*., 2011). Higher content of QC found to be correlated with higher rooting cuttings in many plant species, such as walnut (Cheniany and Ganjeali, 2016), olive (Osterc *et al*., 2007; Izadi *et al*., 2024; Denaxa *et al.,* 2020) and *Eucalyptus grandis* × *E. urophylla* (do Prado *et al*.,2015).

Several studies reported that high polyphenol oxidase (PPO) activity led to a reduction in rooting ability of Kalamata olive cuttings (Ludwig-Müller, 2003), Galega vulgar olive cuttings (Macedo *et al*., 2013), and Kalamata and Arbequina olive cuttings (Denaxa *et al*., 2019). Higher IAAox activity was observed in hard-to-root Kalamata (Denaxa *et al*., 2019). Moreover, lower activities of some enzymes, like IAAox, and higher activities of peroxidase (POD) and catalase (CAT) were accompanied by higher rooting of Pendolino olive cuttings (Kamil *et al*., 2025). Therefore, further studies are needed to improve root induction in olive cv Kalamata cuttings, given their low rooting percentage (Wiesman and Lavee, 1995). Therefore, this investigation aimed to evaluate the efficiency of supplemental IBA combined with either ASC or QC on the rooting efficiency of Kalamata and Wardan cuttings and the associated enzyme activity.

Materials and methods

This experiment was conducted in the Pomology Department nursery at Cairo University's Faculty of Agriculture on two olive cultivars, namely Kalamata and Wardan. It aims to evaluate the effect of supplemental IBA application, with or without ASC and QC.

On 10th December, 600 cuttings (10 treatments × 60 cuttings) of both Kalamata and Wardan olive cultivars were prepared with a length of 20 cm and a diameter of 1–1.5 cm. All prepared cuttings were dipped quickly in IBA at 4000 ppm for 20 seconds before planting in plastic perforated boxes containing a sand: peat moss mixture (4:1) under a plastic tunnel system (Kamil *et al*., 2025). A plastic tunnel is a closed-system alternative for irrigation in places without electricity during cool seasons, where humidity exceeds 85% and night temperatures range from 9-15°C, while daytime temperatures range from 20-25°C. After planting, each cultivar was divided into two groups: one was foliar-treated with IBA at 1000 ppm once a week for 4 weeks (4 applications), and the other was not treated. Also, both groups (foliar-treated or non-treated with IBA) in each cultivar were treated once a week for 4 weeks (4 times) with one of the following treatments: ASC at 50 or 100 ppm, QC at 50 or 100 ppm, or a control. In the same week, foliar treatment with IBA was applied on Sunday, while ASC or QC was applied on Wednesday to avoid interactions between the solutions. IBA and QC were prepared by initially dissolving in ethanol, then diluted with distilled water, while ASC was dissolved in distilled water. All spraying solutions are prepared immediately before application. Four months later, the following measurements were recorded.

Morphological measurements: They include rooting percentage, which was recorded by dividing the rooted cuttings by the total number of cuttings. Also, root number and root length (cm) were recorded.

Enzymatic analysis: One week after finishing foliar treatments, 10 cuttings from each treatment were taken and placed in a refrigerator (-30 °C) for the following.

Enzyme extraction: A sample from the base of the cuttings was taken after finishing spraying treatments at 5 weeks after planting. The samples were taken and immediately kept (-30 °C). For enzyme extraction, the mixture was prepared in 50 mM K_2SO_4 buffer at pH 7.0, which included 1% PVP, 1 mM PMSF, 0.2 mM EDTA (w/v), and 0.1% Triton X-100 (v/v). Following centrifugation (22,000 g) for 10 min at 4 °C, the supernatant was ready for the enzymatic assay (Ragab *et al*., 2022).

Soluble proteins: The protein assay kit (Bio-Rad) utilized the micro-assay method. Mixing a reaction solution containing 80 µL sample, 200 µL of dye reagent and 720 µL of distilled water. The mixture was vortexed, incubated at room temperature (25°C) for 30 minutes, and the absorbance was measured at 595 nm. The BSA stock solution was used as a standard curve for protein quantification.

Catalase activity (CAT): The reduction in absorbance was measured with a UV-Vis spectrophotometer over 1-3 minutes at 240 nm after mixing the enzyme extract with phosphate buffer (pH 7.0) and H_2O_2 (Aebi, 1984). One unit of CAT activity = a decrease of 0.01 absorbance units per minute at 240 nm.

Peroxidase activity (POD): It was determined by mixing the enzyme extract with phosphate buffer, guaiacol (substrate), and H_2O_2, then measuring the rise in absorbance (Chance and Maehly, 1955). One unit of POD activity = increase in absorbance of 0.01 per minute at 470 nm.

Polyphenol oxidase activity (PPO): It was determined using a phosphate buffer containing catechol, and the increase in absorbance over time was measured. One unit of PPO activity is defined as the quantity of enzyme that causes in a rise in absorbance of 0.01 per minute at 420 nm under the assay conditions (Mayer *et al*., 1965).

Indole-3-acetic acid oxidase activity (IAAox): It was determined by incubating the enzyme extract with IAA and $MgSO_4$ in phosphate buffer. Stop reaction after a specific duration (30 minutes) using an acid reagent ($FeCl_3$-$HClO_3$), and IAAox was measured at 530 nm with a spectrophotometer (Mahadevan and Sridhar, 1982).

Statistical analysis: The treatments were arranged in a split-plot design with two factors. The first factor is IBA in the main plot, and the second factor is spraying substances (ASC, QC, and control) in the subplot. The variances between treatments (ANOVA) were calculated using the MSTAT-C statistical package (Freed *et al*., 1990). Then the least significant values were calculated at the 0.05 level (Snedecor and Cochran, 1989).

Results and discussion

Rooting (%): Table 1 shows the effect of supplemental foliar application of IBA at 0 and 1000 ppm, followed by foliar application of either ASC (50 or 100 ppm) or QC (50, 100 ppm), on the rooting percentage of Kalamata olive cuttings. Foliar application of IBA significantly increased rooting of Kalamata cuttings compared to unsprayed cuttings. Generally, ASC recorded the highest significant rooting percentage compared to QC. Also, the highest rooting percentage for Kalamata cuttings was recorded by ASC alone at 50 ppm (12.1%), followed by ASC combined with foliar IBA application (8.5%).

Table 1 shows the effect of foliar application of IBA at 0 and 1000 ppm, followed by foliar application of either ASC (50 or 100 ppm) or QC (50, 100 ppm), on the rooting percentage of Wardan

Table 1. Effect of IBA, ascorbic acid and quercetin on rooting of Kalamata and Wardan olive cuttings.

Foliar application	Kalamata cuttings		Wardan cuttings	
	Foliar IBA		Foliar IBA	
	0 ppm	1000 ppm	0 ppm	1000 ppm
Control	0.00 f	1.00 ef	14.93 g	6.733 h
50 ppm Ascorbic acid	12.10 a	8.50 b	54.40 a	45.27 b
100 ppm Ascorbic acid	3.50 d	5.27 c	19.17 d	22.57 c
50 ppm Quercetin	0.00 f	0.00 f	16.23 f	16.57 ef
100 ppm Quercetin	2.00 e	0.00 f	17.17 e	17.03 ef

olive cuttings. Generally, foliar application of IBA had a negative effect on the rooting percentage of Wardan olive cuttings, except for IBA + 100 ppm ASC. Also, the highest rooting percentage of Wardan cuttings was recorded by individual ASC at 50 ppm (54.4%), followed by 50 ppm ASC + IBA (45.27%).

Root number: Foliar application of IBA negatively affected the number of roots in Kalamata olive cuttings Table 2. Generally, IBA combined with ASC showed a higher, more significant root number than IBA combined with QC. Also, The highest significant number of roots of Kalamata cuttings was recorded by both ASC at 100 ppm (11.33) and 50 ppm (10.1), then IBA+50 ppm ASC (8.27).

Table 2. Effect of IBA, ascorbic acid and quercetin on the number of roots of Kalamata and Wardan olive cuttings

Foliar application	Kalamata cuttings		Wardan cuttings	
	Foliar IBA		Foliar IBA	
	0 ppm	1000 ppm	0 ppm	1000 ppm
Control	0.00 e	0.00 e	6.73 d	7.43 c
50 ppm Ascorbic acid	10.10 b	8.27 c	6.77 d	9.83 a
100 ppm Ascorbic acid	11.33 a	9.90 b	2.33 e	8.73 b
50 ppm Quercetin	0.0 0e	0.00 e	8.53 b	6.83 d
100 ppm Quercetin	6.53 d	0.00 e	6.63 d	7.63 c

Foliar application of IBA had a positive effect on the number of roots of Wardan olive cuttings (Table 2), except for IBA at 50 ppm QC. Generally, ASC combined with IBA showed a significantly higher number of roots than QC combined with IBA. Also, the highest significant root number was recorded with the supplemental foliar application of IBA followed by ASC at 50 ppm (9.83), than both IBA+100 ppm ASC and 50 ppm QC combined with foliar application of IBA (8.73 & 8.53).

Table 3. Effect of IBA, ascorbic acid and quercetin on root length of Kalamata and Wardan cuttings

Foliar application	Kalamata cuttings		Wardan cuttings	
	Foliar IBA		Foliar IBA	
	0 ppm	1000 pm	0 ppm	1000 ppm
Control	0.00 e	0.00 e	5.53 e	5.43 f
50 ppm Ascorbic acid	5.73 b	5.60 b	7.63 a	7.03 b
100 ppm Ascorbic acid	3.67 c	6.17 a	6.83 c	6.23 d
50 ppm Quercetin	0.00 e	0.0 0 e	5.40 f	4.03 i
100 ppm Quercetin	1.0 0 d	0.00 e	4.13 h	5.13g

Root length: Foliar application of IBA had a positive effect on root length of Kalamata olive cuttings when combined with 100 ppm ASC (Table 3). Generally, IBA combined with ASC showed a higher, more significant root length than IBA combined with QC. Also, the longest significant root length of Kalamata cuttings was recorded with 1000 ppm IBA + 100 ppm ASC (6.16cm), followed by both 50 ppm ASC with or without IBA (5.67& 5.73cm).

Foliar application of IBA had a positive effect on root length of Wardan olive cuttings, except for IBA 50 ppm QC and IBA+50 ppm QC, which had a negative effect (Table 3). Generally, IBA combined with ASC recorded a significantly higher root length than IBA combined with QC. Also, the highest significant root length of Wardan cuttings was recorded by ASC at 50 ppm (7.62cm), followed by 1000 ppm IBA (7.03cm).

The findings highlight the significant role of ASC in stimulating rooting. These results were in line with Rashedy et al. (2021), who reported that supplementary intermittent application of IBA and ASC at 300 ppm after planting cuttings improved rooting %, root number, and root length of Coratina, Manzanello, and Picual olive cuttings. Also, ASC stimulates rooting of *Stewartia pseudocamellia* cuttings after treatment with 1000 ppm IBA (Struve and Lagrimini, 1999). More recently, higher success rates in guava cuttings were recorded after soaking in a mixture containing 5% ASC, followed by treatment with 3000 ppm IBA, compared to those treated only with IBA (El-Sharony *et al*., 2018). Sarrou *et al*. (2014) reported that 352.24 ppm ASC acid alone or in combination with 1000 ppm IBA promoted rooting of Wonderful pomegranate cuttings. ASC alone induced rooting in Acacia leprosa when supplied individually, but not when combined with IBA (Radhi and Hussein, 2020). These results may be due to the fact that ASC is a vital component of the antioxidant plant system, which is responsible for H2O2 scavenging (Hasanuzzaman *et al*., 2020), cell multiplication (Ellya Kka, 2017), and cell elongation (Paciolla *et al*., 2019). Furthermore, ASC promotes rooting by increasing the mitotic index of the root quiescent center and the apical meristem, as well as increasing cellular dimensions, root mitotic divisions, and secondary root production through pericycle cell stimulation and divisions (Kaviani, 2014).

The findings indicate that QC stimulates rooting of Wardan cuttings, whereas it does not affect rooting of Kalamata cuttings. These results were in agreement with Denaxa *et al.* (2020), who reported that the levels of QC, rutin, and chlorogenic acid were associated with better rooting in Arbequina olive cuttings, while an inverse pattern was observed in Kalamata olive cuttings (Denaxa *et al.,* 2020). Also, Denaxa *et al*. (2021) stated that the build-up of endogenous root-promoting substances above the girdled area, such as carbohydrates, phenolic compounds (e.g., QC), and polyamines, might be a stimulus for olive cuttings' rooting. Moreover, a significant correlation between QC concentrations and rooting of chestnut cuttings (Osterc *et al*., 2007). Quercetin-3-O-glucoside and quercetin-3-O-rhamnoside were higher in rooting Eucalyptus gunnii cuttings (Do Prado *et al*., 2015).

Enzyme analysis

CAT activity: Foliar application of IBA significantly increased CAT activity in Kalamata cuttings (Fig. 1A). The highest CAT activity was recorded in 50 ppm ASC with or without IBA.

Individual foliar application of IBA significantly increased CAT activity (Fig. 1B). Generally, ASC increased CAT activity compared to QC. The highest CAT activity was recorded in 50 ppm ASC, followed by IBA+50 ppm ASC, while the lowest was recorded in IBA+50 ppm QC, followed by IBA+100 ppm quercetin.

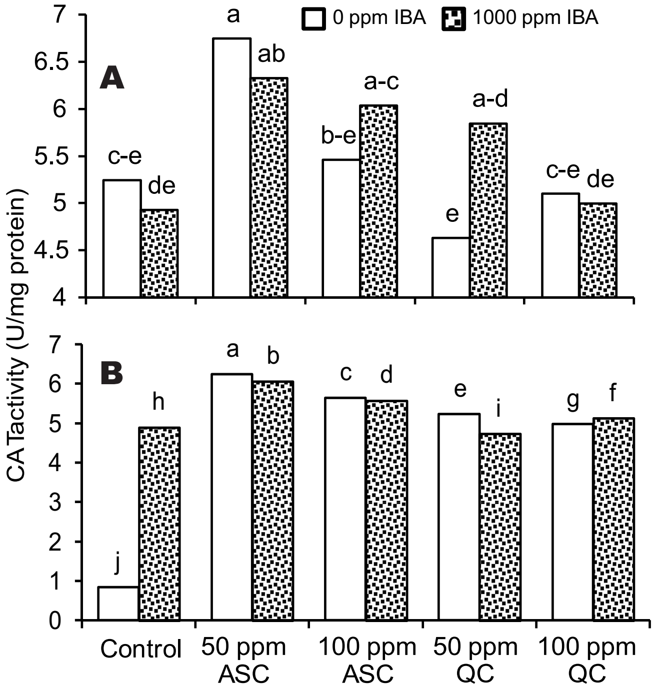

Fig.1 Effect of foliar application of IBA combined with ascorbic acid (50, 100 ppm) or quercetin (50, 100 ppm) on CAT activity of Kalamata (A) and Wardan (B) olive cuttings.

POD activity: Foliar application of IBA significantly increased POD activity in Kalamata olive cuttings (Fig. 2A). Generally, ASC had a positive effect on POD activity of Kalamata olive cuttings, especially when combined with IBA. Also, the highest significant POD for Kalamata cuttings was recorded by the individual ASC at 50 ppm (6.33 U/mg protein), followed by IBA+50 ppm ASC (6.04 U/mg protein).

Foliar application of IBA significantly increased POD activity in Wardan olive cuttings (Fig. 2B). Generally, ASC alone or combined with IBA increased POD activity more than quercetin treatments. Also, the highest significant POD of Wardan cuttings was recorded by individual ASC at 50 ppm alone (6.250 U mg^{-1} protein) or combined with IBA (6.067 U mg^{-1} protein).

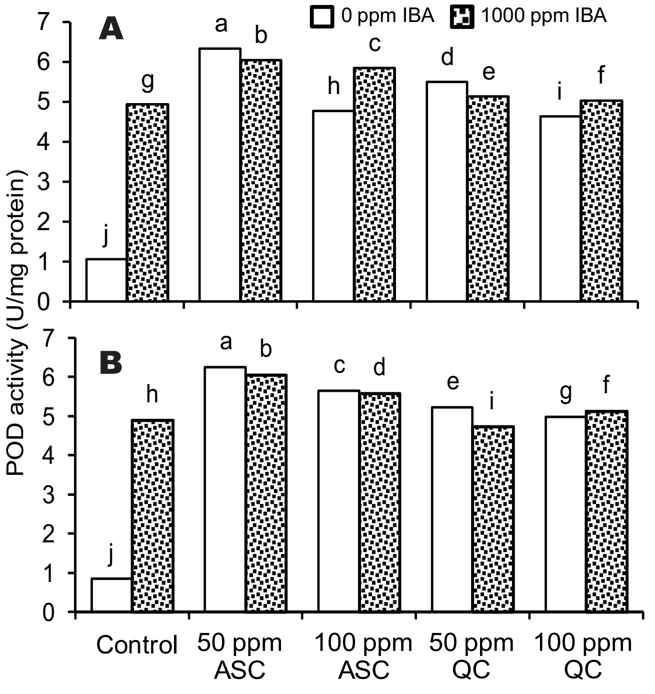

Fig.2 Effect of foliar application of IBA combined with ascorbic acid (50, 100 ppm) or quercetin (50, 100 ppm) on POD activity of Kalamata (A) and Wardan (B) olive cuttings.

PPO activity: Foliar application of IBA tended to increase PPO activity, especially when combined with 100 ppm ASC (Fig. 3A). Generally, ASC decreased PPO activity more than QC. The highest PPO activity was recorded in the control, treated or not treated with IBA. The lowest PPO activity was recorded at 50 ppm ASC alone or combined with IBA.

Individual foliar application of IBA increased PPO activity, and when combined with 50 or 100 ppm ASC, as well as IBA + 100 ppm QC (Fig. 3B), the effect was further enhanced. The highest PPO activity in Wardan olive cuttings was recorded in the treatment with additional IBA, then IBA+100 ppm QC, while the lowest values were recorded in ASC at 50 ppm alone or combined with IBA.

IAAox activity: Generally, additional foliar application of IBA increased IAAox except for IBA+50 ppm QC (Fig. 4A). Also, ASC recorded significantly lower IAAox compared to QC. The highest significant IAAox was recorded in 50 ppm QC without and with IBA, while the lowest IAAox was recorded in 50 ppm ASC with or without IBA.

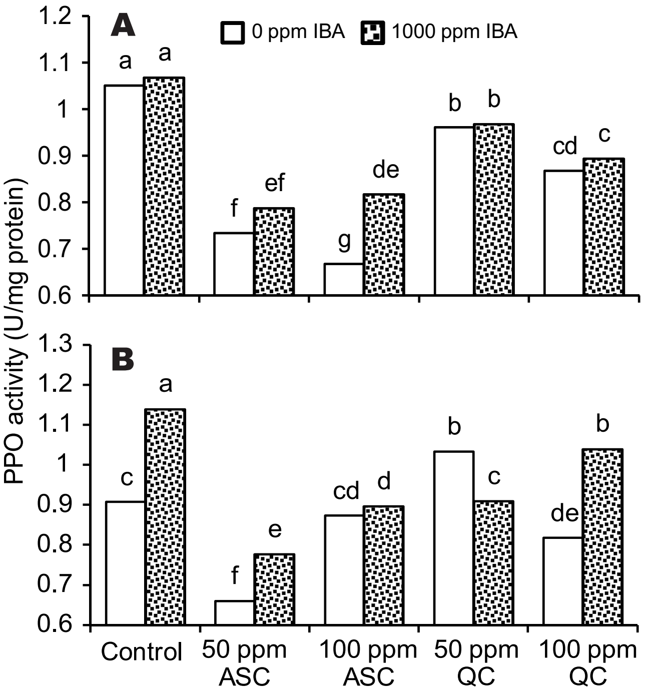

Fig.3 Effect of foliar application of IBA combined with ascorbic acid (50, 100 ppm) or quercetin (50, 100 ppm) on PPO activity of Kalamata (A) and Wardan (B) olive cuttings.

Individual foliar application of IBA decreased IAAox activity in Wardan olive cuttings (Fig. 4B). Generally, ASC is more successful in decreasing IAAox than QC. The highest IAAox was recorded in unsprayed or sprayed IBA treatment, followed by IBA +100 ppm QC.

CAT breaks down H_2O_2, mitigating oxidative stress from reactive oxygen species during rooting, which results from wounding at the cutting's base, promoting healthy root development. While IAAox is involved in the degradation of IAA, the primary auxin in plants. Our findings indicate that ASC plays a greater role in activating the enzyme system than QC, as reflected in increased

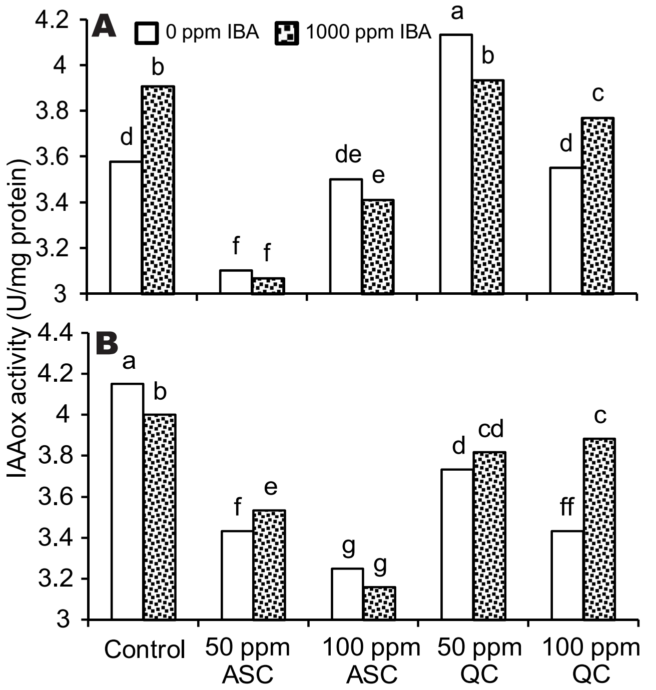

Fig.4. Effect of foliar application of IBA combined with ascorbic acid (50, 100 ppm) or quercetin (50, 100 ppm) on IAAox activity of Kalamata (A) and Wardan (B) olive cuttings.

rooting percentage. These results were in agreement with Kamil *et al.* (2025), who reported that higher rooting of Pendolino olive cuttings was associated with increased CAT and POD and decreased IAAox activity. These results may be due to ASC promoting transverse root formation by acting as an antioxidant, inhibiting IAA-oxidase, and increasing auxin effectiveness in root growth under low pH conditions (Sharma *et al*., 2012; Srivastava and Dubey, 2012). Also, ASC helps in enzyme formation, energy release, photosynthesis, and enhances carbohydrate accumulation in leaves (Barth *et al*., 2006).

QC was effective in stimulating the antioxidant enzyme system in Wardan cuttings. These results may be due to the fact that QC functions as a superior substrate compared to IAA in POD- and oxidase-catalyzed enzymatic reactions (Barz, 1977). Thus, it is likely that the aglycone quercetin actively contributes to adventitious roots formation by sustaining elevated levels of endogenous IAA (Mosella and Macheix, 1979). Polyphenolic compounds like QC and rutin inhibit IAAox and enhance cutting rooting by aiding auxin decarboxylation by POD, while QC and rutin prevent auxin breakdown at cutting bases (Englert *et al*., 1991; De Klerk *et al*., 2011). QC inhibits IAA oxidation and enhances cutting rooting by aiding auxin decarboxylation by POD (Englert *et al*., 1991; De Klerk *et al*., 2011). IBA and QC increased ASC in roots and leaves, activating antioxidant mechanisms, reducing POD activity, and enhancing IAA content, while promoting biochemical changes that lower oxidative stress and boost cell division in Eucalyptus clones (do Prado *et al*., 2015).

In the present study, rooting ability was related to lower PPO and IAAox enzymatic activities in both olive cultivars. These results agree with Denaxa *et al*. (2019), who found that PPO activity was 2 times higher in Kalamata than in Arbequina, while, for the same cultivar, IAAox activity increased 8-fold during the initiation phase (3 days after planting). Also, Ludwig-Müller (2003) reported that the activities of IAAox and PPO were higher in difficult-to-root species than in easy-to-root ones, which may have inhibited the rooting of Kalamata cuttings and partly justified its recalcitrance to root (Bansal and Nanda, 1981). The highest rooting % was accompanied by a 497.4% increase in POD in Kalamata and a 641% increase in the Wardan cultivar.

Supplemental application of 50 ppm ASC was more effective in improving rooting% to 12.1. In Wardan, supplemental IBA negatively affected rooting, but combining IBA+ASC application significantly enhanced it. Rooting correlated with increased POD and CAT activities and decreased PPO and IAAox activities. ASC alone achieved the highest rooting percentage (54.4%) in Wardan cuttings.

The supplemental foliar application of 50 ppm ASC successfully modulated the internal enzymatic environment—specifically by increasing CAT and POD activity while suppressing the auxin-destroying IAAox and PPO. For Kalamata, ASC alone (12.1%) outperformed the combined IBA+ASC treatment, suggesting that for the most difficult varieties, maintaining the internal antioxidant balance is the primary key to triggering adventitious root formation. In contrast, Wardan showed the most benefit from the combined use of IBA and ASC. These results provide a biological basis for adjusting propagation methods to specific olive varieties.

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Received: December, 2025; Revised: January, 2026;
Accepted: January, 2026