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BIOCHAR, SILVER NANOPARTICLES AND IBA HORMONE FOR ENHANCING ROOTABILITY OF SOME CULTIVARS OF OLIVE CUTTINGS

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ABSTRACT: In the current study, a novel application of using nano-materials and organic derived-biochar has been applied to promote some olive cuttings rooting compared to traditional materials like IBA. It was expected that a smarter practise of these substances in fruit production could be achievable to solve cuttings rooting problems. However, interactions of both materials with olive cuttings are required to be under stood prior to wide-ranging recommendation. The present study assists to explore the influence of silver (Ag) nanoparticles (NPs) and IBA treatments with or without biochar on enhancing rootability of Kalamata and Picual olive cuttings. Different concentrations of AgNPs (0, 1, 5, 10, 20 and 40 ppm), IBA (3000, 4000 and 5000 ppm) and olive pomace derived-biochar (0, 10, 20, 30, and 40 grams per each pot) have been applied to olive cuttings. After the treatments were applied to the basal end hardwood cuttings were immediately planted in polyethylene pots (10 cm × 20 cm) filled with a sand : peatmoss at the ratio of 1:1 and kept in a shade house and 80-90% relative humidity and high basal temperature (24 °C). The results showed superior positive effect of biochar addition at 40 g per pot on percentage of rooted cuttings, number of roots per cutting, root and branch length and root fresh weights as compared with control in case of Kalmata and Picual olive *cvs.*in both seasons. The AgNPs at 20 ppm enhanced obviously the rooted cuttings percentage and number of roots per cutting, while IBA at 3000 ppm was most effective on root and branch length and root fresh weights as compared with the other treatments for Kalmata and Picual olive *cvs.* in both seasons. This study open a new door for investigating the effect of the novel applications of AgNPs and biochar in different fruit cuttings propagation taking into account that each fruit species require an individual experimental studies to determine the promising additions of both treatments.

Key words: Biochar; silver; nanoparticles; Indole-3-butyric acid; olive cuttings; Kalmata, Picual and Manzanillo*cvs.*

INTRODUCTION

Olive (*Olea europaea* L.) is an important ancient and economical crop in arid and semiarid area of the Mediterranean region, especially in Egypt **(Roussos *et al.*, 2010; Isfendiyaroglu and Ozeker, 2009).** Growth regulators (hormones) are common use widely for inducing root formation in mist propagation system and IBA is considered as the best artificial hormone which used in this concern. **(Khajehpour *et al.*, 2014; Muller *et al.*, 2005).** Olive cultivars could be vegetativly propagated using different methods *i.e.* grafting and budding **(Hartmann *et al.*, 1990)** but semi-hardwood cuttings are one of the cheap and easiest methods of vegetative propagation **(Dvin *et al.*, 2011; Hartmann *et al.*, 2002).** Previous researchers reported that the biggest problem in propagation of some olive cultivars is the low ability of regeneration leading to low percentage of rooting **(Fabbri *et al.*, 2004).** Root in ability of olive cuttings is influenced by a number of factors related to cultivars **(Turkoglu and Durmus, 2005; Fouad *et al.*, 1990),** age of source tree and prelevement date of cuttings **(Sebastiani and Tognetti, 2004; Ahmed *et al.*, 2002),** type of cuttings **(Turkoglu and Durmus, 2005)**, rooting media **(Isfendiyaroglu and Ozeker 2009; Awan *et al.*, 2003)** and the treatment concentration of auxin-like compounds **(AslMoshtaghi and Shahsavar, 2011; Hartmann *et al.*, 2002).**

It is well known that several methods have been used to promote cuttings root ability include chemical, biological and mechanical approaches **(Barabanov *et al.*, 2018)**. Creation and application of nano sized particles of numerous substances is known as one of the most rapidly developing trends in modern nanotechnology **(Cornier *et al.*, 2017)**; in the agriculture industry, engineered nanoparticles have been serving as "nano carrier", containing herbicides, chemicals, or genes, which target particular plant parts to release their content **(Zakaria *et al.*, 2015; Lian *et al.*, 2012; Wu *et al.*, 2011).** Among these techniques, root development in plant cuttings is quite tedious in spite of using rooting hormones due to two main factors: different rooting abilities and root growth-deteriorating phyto pathogens eventually resulting in a huge loss in horticulture **(Steve Dreistadt, 2001).** In order to solve these issues, a unique approach (nanoparticles) was devised to utilize the rooting hormone-stabilized silver nanoparticles both as root promoters. Silver nanoparticles (AgNPs) are one of the most commonly used manufactured nanoparticles in consuming products where they are increasingly used for their antimicrobial properties **(Solaiman *et al.*, 2017).** Recently, there have been only a few studies of the influence of AgNPs on higher plants **(Cvjetko *et al.*, 2017; Thuesombat *et al.*, 2014; Yin *et al.*, 2012)**, but these have steadily shown that AgNPs have different effects (negative or positive) on cuttings root ability and plant growth. **Thangavelu *et al.* (2016)** succeeded in AgNPs synthesis, using two auxin rooting hormones of Indole-3-acetic acid and Indole-3-butyric acid as a reducing cum stabilizing agent for a dual mode like root enhancer and pathogen destroyer on the target site. The action duality of hormone-stabilized AgNPs was manifested to threefold enhanced root growth compared to controls and it increased the rooting capabilities against root growth inhibiting phyto pathogens.

Biochar (BC) is a highly stable form of carbon manufactured by burning organic materials (such as organic waste, manure, crop residues) under oxygen-limited conditions (*i.e.* biomass pyrolysis process). It has been reported that biochar could be stable and remain in soil for several years **(Ascough *et al.*, 2009; Lehmann and Joseph, 2009).** Recently, it used as a soil amendment that can act as a carbon sink in arable land as well as improve soil fertility, thus improving crop growth and yield **(Atkinson *et al.* 2010; Major *et al.*, 2010; Asai *et al.*, 2009; Lehmann and Steiner, 2009; Chan *et al.*, 2007).** For instance, biochar amendment significantly increased the total absorptive surface area of the root system of *Poncirus trifoliate* L. seedlings **(Changxun *et al.*, 2016)**. Moreover, there have been very few studies reporting the influence of biochar on plant early stage growth such as cutting root ability. However, BC may contain unwanted substances such as hydrocarbons (PAHs), phenolic compounds and metals that may pose significant risk to plants, microbes and humans **(Thies and Rillig, 2009)**. Some compounds in BC have the potential to either inhibit or stimulate seed germination and seedling growth as they contain essential nutrients for plant **(Gaskin *et al.*, 2008)**. Biochar has been reported to either increase **(Chan *et al.*, 2008)** or decrease **(Deenik *et al.*, 2010)** plant growth and yield.

According to our knowledge, there are no previous studies that have tried to combine AgNPs and biochar for promoting olive cuttings root ability. Therefore, the main objective of this work was to determine whether silver nanoparticles and biochar treatments could increase root ability of hardwood cuttings of Kalamata and Picual olive cultivars compared to traditional method (IBA hormone).

MATERIALS AND METHODS

This present study was carried out in a nursery of the Experimental farm of the Faculty of Environmental and Agricultural Sciences, Arish University, North Sinai Governorate, Egypt, to display the effect of silver nanoparticles, IBA hormone and biochar treatments on the rooting ability of three olive cultivars during 2018 and 2019 seasons. The hardwood cuttings of olive *cvs.* Kalamata, Picual and Manzanillo taken from one-year-old branches were used in January of each season about 15-18cm in length of cuttings with two pairs of leaves were collected from a 20-years-old olive orchard. Different concentrations of AgNPs (1, 5, 10, 20 and 40 ppm), Indole-3-butyric acid (IBA) solution at 3000, 4000 and 5000 ppm as well as control treatment, and olive pomace derived-biochar (0, 10, 20, 30, and 40 g per each plastic pot) have been applied to olive hardwood cuttings. Water was treated as control. The basal end of cuttings (2-3 cm) were dipped in AgNPs and IBA solutions for 10 seconds following the recommendations of **De Oliveira *et al.* (2003).** After the treatments were applied to the basal end hardwood cuttings were immediately planted in polyethylene pots (10 cm × 20 cm) filled with a sand : peatmoss at the ratio of 1:1 and kept in a shade house and 80-90% relative humidity and high basal temperature (24 °C).

Silver nano particle preparation

Silver nanoparticles were purchased from Agriculture Research Centre (ARC) Giza, Egypt. The AgNPs was synthesized as described by **Vigneshwaran *et al.* (2006).** Briefly, silver nanoparticles were produced using green chemistry method where 1.0 g of soluble starch was dissolved in 100 ml of deionized water. One ml of a 100 mM$AgNO_3$ was added and well stirred. This mixture was autoclaved at 15 psi pressure, 121 ºC for 5 min. The resulting colour of the nano particle suspension solution was clear yellow indicating the formation of silver nanoparticles **(Vigneshwaran *et al.*, 2006).** Silver nanoparticles were brought from ARC at concentration of 100 mgl^{-1} and at diameter size less than 100 nm. To check the nano size of AgNP provided from ARC, scanning electron microscope (SEM; Quanta 450 FEG-ESEM, FEI Company) was used as seen in **Figure (1)** which confirm a size of less than 100 nm.

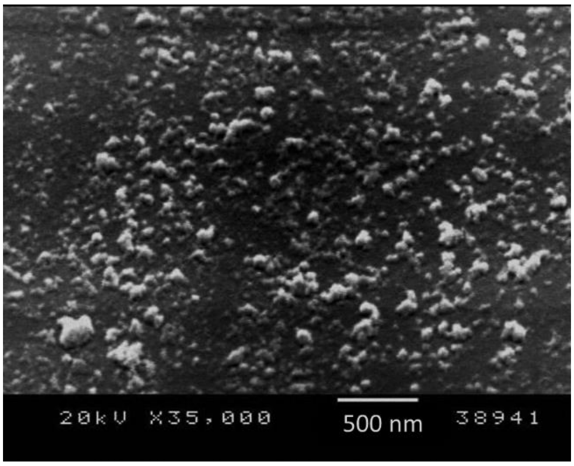

Figure 1. SEM image of silver nanoparticles that confirm the size of less than 100 nm.

Biochars preparation and characterization

Olive pomace wastes were collected from experimental oil extractor at Faculty of Environmental Agricultural Sciences, Arish University, North Sinai Governorate, Egypt and used to produced biochar. A known quantity of air dried material was taken in closed crucible and heated in muffle furnace at 450 °C at 60 min as described by **Vijayanand *et al.* (2016).** The morphological features of the produced biochar were investigated by scanning electron microscope (SEM; Quanta 450 FEG-ESEM, FEI Company). **Figure (2)** showed smooth surfaces with different porosity sizes. The pore sizes were not uniform and were in the range of tens of nano- to micro-meters. Produced biochar was chemically characterized; biochar pH was measured using pH meter (Model pH 209, HANNA Instruments, UK) in water : solid ratio of 1: 2.5. Total metal concentrations were measured in acid digested biochar (aqua regia; 3:1 mixture of concentrated HCl and HNO_3) using atomic absorption spectrophotometry (SHIMADZU AA-6800). Biochar organic matter was estimated using the oxidizabledichromate method **(Walkley and Black, 1934)**. **Table (1)** summarized chemical characteristics of olive pomace-derived biochar.

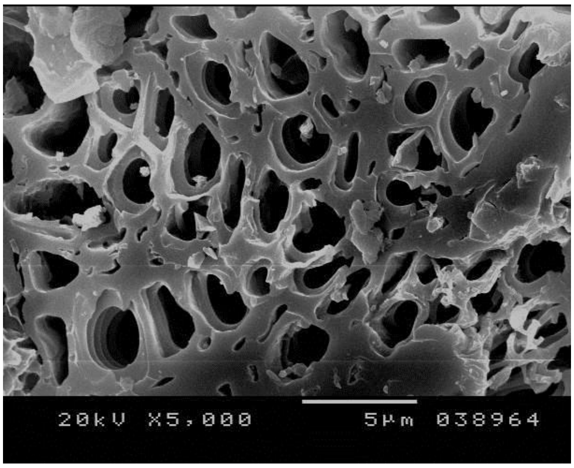

Figure 2. Scanning electron micrograph of biochar produced at 450 ºC.

Table 1. Summary of chemical characteristics of olive pomace-derived biochar

Biochar	pH (1:2.5)	OM (%)	Zn	Fe	Na	Mg	K	Ca
			(g.kg^{-1})					
	8.94	52.9	0.022	0.41	0.06	3.05	13.2	23.1

Measurements

Cuttings were removed from soil 90 days after treatments, and data were recorded on the percentage of rooted cuttings (%), number of roots per cutting, root fresh weights (g), root and branch length (cm) were measured.

Statistically Analysis

The experiment was included nine main treatments (control treatment, five AgNPs treatments and threeIBA concentrations) as well as sub-main treatments (olive pomace derived-biochar applications). The treatments were arranged as a factorial experiment in a randomized complete block design (RCBD) at 3 factors split plots with three replicates and each replicate was represented by five cuttings. Data obtained in this study were recorded and statistically analyzed using MS-TATC software and the graphs were drawn by MS-Excel software **(Steel and Torrie 1980).** Duncan´s multiple range test was used to separate treatment means to found significantly different in the analysis of variance **(Duncan, 1955).**

RESULTS AND DISCUSSION

Rooted cuttings (%)

With regard to the effect of biochar (BC) applications, data in **Table (2) and Figure (3)** indicated that biochar (BC) at 40 g per pot significantly increased the rooted cuttings percentage (34.22 and 37.39%) and (68.48 and 67.89%) for Kalmata and Picual olive *cvs.* in both seasons, respectively. While, control treatment induced less simulative effect in the rooted cuttings percentage (18.24 and 18.76%) and (46.95 and 47.89%) for two olive cultivars in the first and second season, respectively. It was observed that the percentage of rooted cuttings increased significantly with increase in Ag NPs concentration. The AgNPs at 20 ppm enhanced obviously the rooted cuttings percentage (50.29 and 52.51%) and (84.11 and 86.30%), followed by IBA concentration of 3000 ppm (46.95 and 45.77%) and (68.51 and 81.34%) for Kalmata and Picual olive *cvs.*in both seasons, respectively. On the contrary, control treatment recorded least percentage of rooted cuttings percentage (10.75 and 11.34%) and (29.91 and 30.85%) for Kalmata and Picual olive *cvs*. in both seasons, respectively. Concentration of 40 ppm of silver nanoparticles showed statistically significant inhibition on rooted cuttings percentage (%). Thus, silver nanoparticles can be reported with minimal toxicity on the tested cuttings, this is a good evidence for demonstrating that Kalmata and Picual olive *cvs.* respond to add AgNPs in a limited range, above which toxic levels are reached causing subsequent declines in rooting. These results go in line with those reported by **Thangavelu *et al.* (2016) and Dharanivasan (2016)** they reported that the action duality of hormone-stabilized AgNPs was manifested to three-fold enhanced root growth compared than controls and it increased the rooting capabilities against root growth inhibiting phyto pathogens. Concerning the interaction effect between Ag nanoparticles and biochar applications, **Table (2)** reveals that IBA hormone with biochar shows lower effect compared to AgNPs with biochar rates. The highest rooted cuttings percentage was obtained with AgNPs at 20 ppm by biochar at 40 g per pot (55.54 and 60.54%) and (91.86 and 92.56 %) for Kalmata and Picual olive *cvs.* in both seasons, respectively. In the meantime, the least one was given by untreated cuttings (control) (3.45 and 4.16%) and (16.72 and 17.11%) for two cultivars in both seasons. The other interactions revealed in between effects. The response of Picual olive *cv.* was superior than Kalmata *cv.* to rooted cuttings percentage in both seasons.

Number of roots per cutting

Concerning, the effect of biochar (BC) applications on number of roots per cutting, data in **Table (3) and Figure (4)** revealed that biochar (BC) at 40 g per pot recorded the highest number of roots, followed by biochar (BC) at 30 g per pot in both seasons. On the other hand, control treatment caused a significant reduction in number of roots for Kalmata and Picual olive *cvs.* in both seasons. Our results suggest that the positive effects of biochar on root ability can be attributed to the substantial augmentation of media fertility, and increased soil pH. These results agree with those reported by **Changxun *et al.* (2016)** who reported that biochar facilitates citrus to grow more lateral roots and thus increase the root surface area. Data in **Table (3) and Figure (4)** indicated that silver nanoparticles (AgNPs) treatment at 20 ppm enhanced obviously the number of roots (20.10 and 20.35) and (22.17 and 21.74) for Kalmata and Picual olive *cvs.* in both seasons, respectively. While, the untreated cuttings gave least number of roots (8.74 and 7.37) for Kalmata and (8.90 and8.01) for Picual in both seasons, respectively. These results are in the same line of **Thangavelu *et al.* (2016) and Salama (2012)** who reported that increasing concentration of silver nanoparticles has led to an increase in root growth parameters of the two tested crop plants. Regarding the interaction effect, **Table (3)** shows that the AgNPs at 20 ppm with biochar (BC) at 40 g per pot recorded the highest number of roots per cuttingfor Kalmata and Picual olive *cvs.* in both seasons, respectively. On the other hand, the least values given by untreated cuttings (control). The other interactions came in between. The mean finding of this part is that AgNPs with biochar at the highest concentrations showed promising results over IBA applications on number of roots per cutting of Kalmata and Picual olive *cvs*. Picual olive *cv.* was superior on Kalmata *cv.* to root numbers per cutting in both seasons.

Table 2. Rooted cuttings percentage (%) of olive cultivars treated by Ag nanoparticles, IBA and biochar applications at different rates (2018 and 2019 seasons)

Cultivars	Biochar (g/pots)	Control	AgNPs (ppm)					IBA (ppm)			Mean of biochar rates
		0	1	5	10	20	40	3000	4000	5000	
Season 2018											
Kalmata cv.	**0 g**	3.45 w	8.63 uv	19.59 op	23.68lmn	44.11 e	28.44 j	18.56 p	11.45 t	6.28 v	**18.24C**
	10 g	8.87 uv	9.92 u	22.92 mn	24.86 l	45.87de	28.72 j	47.33 d	22.33 n	11.62 t	**24.72BC**
	20 g	11.72 t	10.81tu	23.61lmn	26.11kl	50.92 c	33.03g	52.53 bc	24.78 l	12.90 st	**27.38B**
	30 g	12.25 st	13.84 s	24.55 lm	27.67jk	54.99ab	37.00 f	53.74 b	26.77jkl	16.51 r	**30.04AB**
	40 g	17.44 q	20.34 o	26.27 kl	30.44 h	55.54 a	44.03 e	54.57 ab	29.44 i	24.93 l	**34.22A**
Mean of AgNPs and IBA		**10.75G**	**12.71F**	**23.39D**	**26.55C**	**50.29A**	**34.24B**	**46.95AB**	**22.95CD**	**14.45E**	
Picual cv.	**0 g**	16.72 x	40.80 r	56.23lmn	68.11 h	75.74ef	54.69mno	58.47 l	28.68 v	22.12 w	**46.95E**
	10 g	27.98vw	43.05 q	57.92 lm	70.15 g	78.01de	57.42 lm	61.39 kl	55.93mn	40.92 r	**54.75D**
	20 g	31.37 u	43.48 q	64.29 ijk	77.87def	86.59 b	63.74 jk	68.15 h	62.08 jkl	49.52 o	**60.79C**
	30 g	35.54 t	44.35 p	66.86 i	84.10 cd	88.33ab	65.65 ij	73.60 f	67.04 hi	53.48no	**64.33B**
	40 g	37.95 s	49.46 o	69.53 gh	85.78 c	91.86 a	70.25 g	80.96 d	73.75 f	58.82 l	**68.48A**
Mean of AgNPs and IBA		**29.91F**	**44.03E**	**62.97CD**	**77.20B**	**84.11A**	**62.35CD**	**68.51C**	**57.50D**	**44.97E**	
Season 2019											
Kalmata cv.	**0 g**	4.16 x	7.68 w	17.63 r	21.79opq	44.99fg	31.00 l	17.88 r	13.24	18.33qr	**18.76D**
	10 g	7.45w	8.83vw	20.63pq	22.87op	46.79ef	31.31 kl	45.59efg	24.49 no	19.45 q	**25.26C**
	20 g	11.06 u	9.62 v	21.25opq	24.02 no	51.94cd	33.69 jk	50.61 d	32.09jkl	21.46opq	**28.42BC**
	30 g	15.48 s	12.32 t	22.10 op	25.46mno	58.29 b	40.33 h	54.66 c	34.65 j	27.47mn	**32.31B**
	40 g	18.54qr	18.11qr	23.64nop	28.01 m	60.54 a	47.99 e	60.12 a	38.12 i	43.48 g	**37.39A**
Mean of AgNPs and IBA		**11.34G**	**11.31G**	**21.05F**	**24.43E**	**52.51A**	**36.86C**	**45.77B**	**28.52D**	**24.04E**	
Picual cv.	**0 g**	17.11 x	37.20stu	50.61 p	62.66 k	77.25 e	55.27 n	62.33 k	37.11 stu	31.48 w	**47.89D**
	10 g	36.22 u	38.32st	52.13 o	64.54 j	82.22 c	62.59 k	77.91 de	53.81 no	45.65 q	**57.04C**
	20 g	31.30 w	38.70st	57.86 mn	71.64 fg	88.33abc	66.29 i	86.48bc	59.73 lm	55.23 n	**61.73B**
	30 g	32.84 v	39.48 s	60.17 l	77.37 de	91.16ab	68.23 h	88.21abc	64.51 j	58.44lmn	**64.38AB**
	40 g	36.78 tu	42.24 r	62.58 k	78.92 d	92.56 a	72.05 f	91.74 ab	70.96 g	63.19 jk	**67.89A**
Mean of AgNPs and IBA		**30.85G**	**39.19F**	**56.67DE**	**71.03B**	**86.30A**	**64.89C**	**81.34AB**	**57.22D**	**50.60E**	

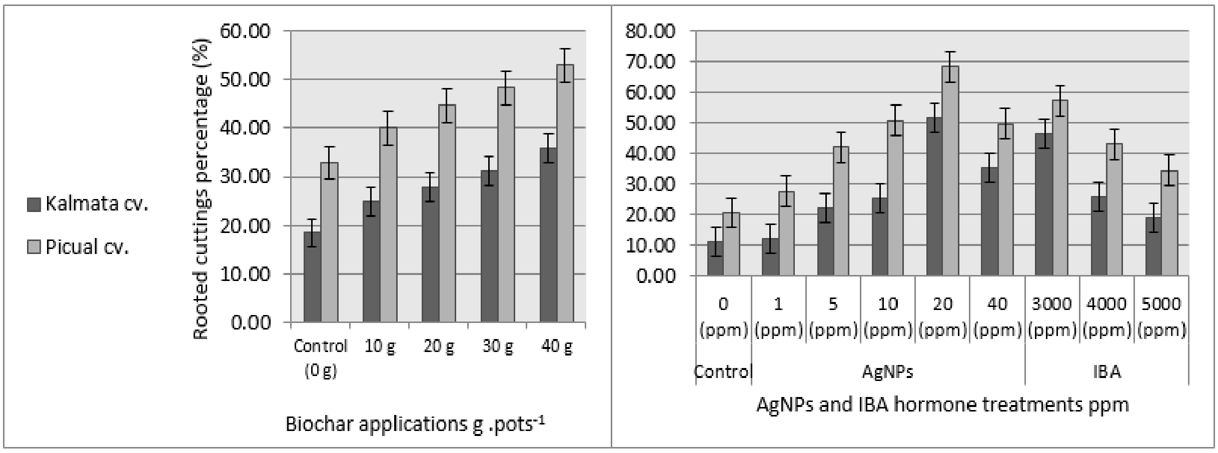

Figure 3. Rooted cuttings percentage (%) of Kalamata and Picual olive cultivar cuttings treated by Ag nanoparticles, IBA and biochar applications at different rates. Error bars represent standard errors of triplicates.

Table 3. Number of roots per cutting of olive cultivars treated by Ag nanoparticles, IBA and biochar applications at different rates (2018 and 2019 seasons)

Cultivars	Biochar (g/pots)	Control 0	AgNPs (ppm) 1	5	10	20	40	IBA (ppm) 3000	4000	5000	Mean of biochar rates
Season 2018											
Kalmata cv.	**0 g**	5.45s	9.37pq	10.07 o	13.29ijk	17.11de	13.42ij	15.23g	7.31qr	6.45r	**10.86D**
	10 g	8.12q	10.78mno	11.78lm	13.95hij	18.48cde	14.23hi	17.51 d	9.87op	9.45pq	**12.69C**
	20 g	9.33pq	11.43m	12.14klm	14.65gh	20.51bc	16.36efg	18.74 cd	11.74lm	9.81op	**13.86B**
	30 g	9.74opq	12.34kl	12.62jkl	15.53fg	22.15ab	16.69ef	19.49 c	12.80jk	10.59no	**14.66AB**
	40 g	11.07mn	14.44ghi	13.50ij	17.08def	22.23a	17.19de	21.24abc	14.98gh	11.06mn	**15.87A**
Mean of AgNPs and IBA		**8.74H**	**11.67F**	**12.02E**	**14.90D**	**20.10A**	**15.58C**	**18.44B**	**11.34F**	**9.47G**	
Picual cv.	**0 g**	7.44v	8.49tu	11.05qr	14.15mn	19.45ef	18.61g	17.11j	13.23no	10.95qr	**13.39D**
	10 g	8.11u	8.74stu	11.38q	14.57m	20.42d	19.17f	17.97hij	16.54k	12.59p	**14.39CD**
	20 g	8.74stu	8.83stu	12.63p	16.18kl	22.67b	21.28cd	19.94de	18.36gh	15.24l	**15.98C**
	30 g	9.34st	9.63s	13.14o	17.47ij	23.35ab	21.70bc	21.54bcd	19.83def	16.46k	**16.94B**
	40 g	10.86r	12.23pq	13.66n	18.00hi	24.98a	22.57b	23.69ab	21.81bc	18.10ghi	**18.43A**
Mean of AgNPs and IBA		**8.90H**	**9.58G**	**12.37F**	**16.07D**	**22.17A**	**20.67B**	**20.05B**	**17.95C**	**14.67E**	
Season 2019											
Kalmata cv.	**0 g**	4.85s	8.34pqr	10.00no	12.23k	17.45ef	14.63g	16.60f	11.12lm	7.03qrs	**11.36E**
	10 g	6.11rs	9.59op	10.96lmn	12.84j	18.85cd	15.51fg	17.44ef	11.76 l	9.45opq	**12.50D**
	20 g	7.38qr	10.17mno	12.77jk	13.48hi	20.92b	17.83e	19.36c	12.27k	10.49mn	**13.85C**
	30 g	8.67pq	10.98lmn	13.78h	14.29gh	21.84ab	18.19de	20.91b	13.25hij	12.11kl	**14.89B**
	40 g	9.85nop	12.85j	14.62g	15.72fg	22.67a	18.73cde	22.28a	14.58g	13.00ij	**16.03A**
Mean of AgNPs and IBA		**7.37F**	**10.39E**	**12.43D**	**13.71C**	**20.35A**	**16.98B**	**19.32AB**	**12.60D**	**10.42E**	
Picual cv.	**0 g**	6.70v	7.56uv	9.95qrs	13.02n	20.34efg	16.56k	16.55k	12.28o	10.77pqr	**12.64E**
	10 g	7.30uv	8.13tu	10.24qr	14.19m	20.75e	18.11i	20.69ef	17.81ij	15.62 l	**14.76D**
	20 g	7.87tuv	8.77st	11.37opq	15.31lm	21.70cd	19.45g	22.96bc	19.76fg	16.55k	**15.97C**
	30 g	8.41stu	9.11rst	11.82op	17.28ijk	22.19bcd	20.91de	23.42abc	21.35cde	17.05jk	**16.84B**
	40 g	9.77rs	10.88pq	12.30o	18.34hi	24.36a	21.11cde	23.73ab	23.48abc	18.76h	**18.08A**
Mean of AgNPs and IBA		**8.01F**	**8.89F**	**11.14E**	**15.63D**	**21.75A**	**19.23B**	**21.58A**	**18.94C**	**15.75D**	

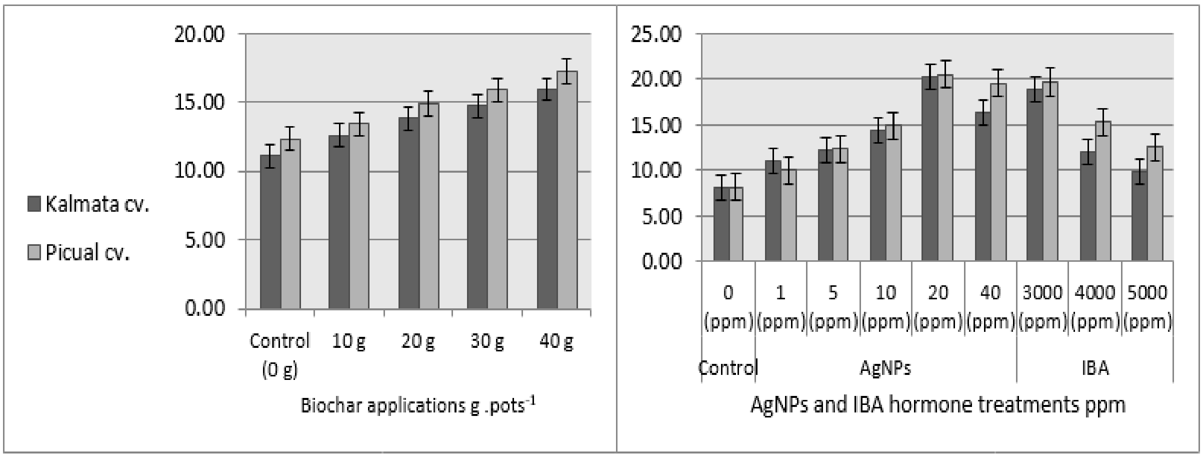

Figure 4. Roots number of Kalamata and Picual olive cultivar cuttings treated by Ag nanoparticles, IBA and biochar applications at different rates. Error bars represent standard errors of triplicates.

Root and branch length (cm)

Concerning, the effect of biochar (BC) applications on root and branch length (cm), data in **Tables (4 & 5) and Figures (5 & 6)** showed that biochar (BC) applications significantly increased root and branch length. The highest values in the both seasons were recorded by biochar (BC) at 40 g per pot than untreated cuttings (control) for Kalmata and Picual olive *cvs.* It seems that biochar at high rates showed a better treatment compared with low rates. Moreover, statistical analysis of root and branch length among different treatment showed high variability in case of biochar treatment. This positive effect may be due to the biochar high content of organic matter which reflects in soil fertility and increased soil pH. Our results confirmed the positive role of biochar on root architecture and growth. These results tend to agree with those reported by **Abo-Ogiala (2018)** who reported that biochar increased root length and plant performance of Volkamer Lemon (*C. volkameriana*, Tenxpasq.) under saline condition. Also, **Amendola *et al.*, (2017)** who found that mean fine root diameter was significantly higher in biochar-treated plants than in control on grapevine explants. Unlike biochar, IBA at 5000 ppm showed low root and branch length **Tables (4 & 5) and Figures (5 & 6)**. Root and branch length became progressively lower with IBA application. Root and branch length were positively correlated with decreasing the concentration of IBA. The IBA hormone at 3000 ppm showed to be the most effective on root and branch length as compared with the other treatments for Kalmata and Picual olive *cvs.* in both seasons.Similar observations were reported by **Kurd *et al.* (2010)**who reported that the maximum average number of roots per cutting and average root length were recorded with IBA treatment, and **Khajehpour *et al.* (2014)** who found that 3000 ppm IBA increased the number of roots and branch length, and root: shoot fresh weight of Manzanilla olive Cuttings. Regarding, the interaction effect of silver nanoparticles (AgNPs) and IBA hormone treatments and biochar (BC) applications, data presented in **Tables (4 & 5)** show that IBA treatments by biochar applications were significantly interactive for root and branch length. The IBA treatment at 3000 ppm by biochar (BC) at 40 g per pot had higher root and branch length, while the untreated cuttings (control) gave the least values for Kalmata and Picual olive *cvs.* in both seasons, respectively. The other interactions came in between. Picual olive *cv.* was superior on Kalmata *cv.* to root and branch length (cm) in both seasons.

Table 4. Root length (cm) of olive cultivars treated by Ag nanoparticles, IBA and biochar applications at different rates (2018 and 2019 seasons)

Cultivars	Biochar (g/pots)	Control 0	AgNPs (ppm) 1	5	10	20	40	IBA (ppm) 3000	4000	5000	Mean of biochar rates
Season 2018											
Kalmata cv.	**0 g**	2.33s	5.13pq	5.77opq	6.11o	8.07jk	10.32fg	11.58de	8.12ijk	6.06o	**7.05C**
	10 g	3.27r	5.89op	6.17o	6.42mno	8.39ij	10.83efg	13.32c	8.53i	6.97lmn	**7.75BC**
	20 g	3.91qr	6.25no	6.36no	6.99lmn	9.32hi	12.45d	14.78b	9.46hi	7.74kl	**8.58B**
	30 g	4.73pqr	6.75mn	6.61mn	7.41klm	10.06gh	13.95bc	15.96ab	10.22fgh	9.90ghi	**9.51AB**
	40 g	7.08lm	7.11lm	7.90jkl	8.15ijk	10.16gh	15.60ab	16.76a	11.24def	10.99ef	**10.67A**
Mean of AgNPs and IBA		**4.27F**	**6.38E**	**6.40E**	**7.02DE**	**9.20C**	**12.83B**	**14.48A**	**9.51C**	**8.33D**	
Picual cv.	**0 g**	6.19q	8.36op	9.03no	9.84lm	10.95jk	13.53g	15.23ef	11.12j	11.09j	**10.59E**
	10 g	7.92pq	8.61o	9.30mn	10.14klm	11.28ij	14.21fg	15.99de	12.12hij	11.31ij	**11.21D**
	20 g	8.04opq	8.69o	10.32kl	11.25ij	12.52h	15.77def	17.75c	13.45gh	13.69g	**12.39C**
	30 g	9.01no	9.74lmn	10.74jkl	12.15hij	12.77h	16.24d	19.17b	14.53fg	14.78efg	**13.24B**
	40 g	9.12mno	11.39ij	11.17j	12.39hi	13.28gh	17.38c	21.09a	15.98de	16.26d	**14.23A**
Mean of AgNPs and IBA		**8.06H**	**9.36G**	**10.11F**	**11.15E**	**12.16D**	**15.43B**	**17.85A**	**13.44C**	**13.43C**	
Season 2019											
Kalmata cv.	**0 g**	3.61r	4.56pq	5.19opq	5.62op	8.23klm	11.25h	10.16ij	10.00ijk	8.56kl	**7.47E**
	10 g	3.98qr	5.25opq	5.56op	5.90no	8.56kl	11.80gh	15.75ef	10.20hij	9.84jk	**8.54D**
	20 g	4.11q	5.56op	5.72nop	6.43mno	9.50k	13.58fg	17.48cd	13.36fgh	9.94ijk	**9.52C**
	30 g	6.34mno	6.01no	5.95no	6.82mn	10.26hij	17.20d	18.88b	14.43f	11.73gh	**10.63B**
	40 g	6.44mno	7.03m	6.37mno	7.50lm	10.37hi	18.09bc	20.77a	17.72bcd	15.87e	**12.24A**
Mean of AgNPs and IBA		**4.90G**	**5.68F**	**5.76F**	**6.46EF**	**9.38E**	**13.99B**	**16.61A**	**12.77C**	**11.56D**	
Picual cv.	**0 g**	5.49q	7.44op	8.13mno	9.05 l	11.17ij	14.75def	14.99de	10.51j	8.92lm	**10.05C**
	10 g	6.33pq	7.66no	8.37mn	9.32kl	11.50hi	15.49d	15.94cd	12.09h	9.37kl	**10.67C**
	20 g	7.05opq	7.74no	9.29kl	10.35j	12.77gh	16.40c	17.69b	13.42f	11.33hij	**11.78BC**
	30 g	7.56nop	8.67lmn	9.66jkl	11.18ij	13.02g	16.57bc	18.05ab	13.33fg	11.79hi	**12.20B**
	40 g	9.51jkl	10.14jk	10.05jk	11.40hij	13.55f	17.73b	19.56a	14.66ef	12.96g	**13.28A**
Mean of AgNPs and IBA		**7.19G**	**8.33F**	**9.10E**	**10.26D**	**12.40C**	**16.19B**	**17.25A**	**12.80C**	**10.87D**	

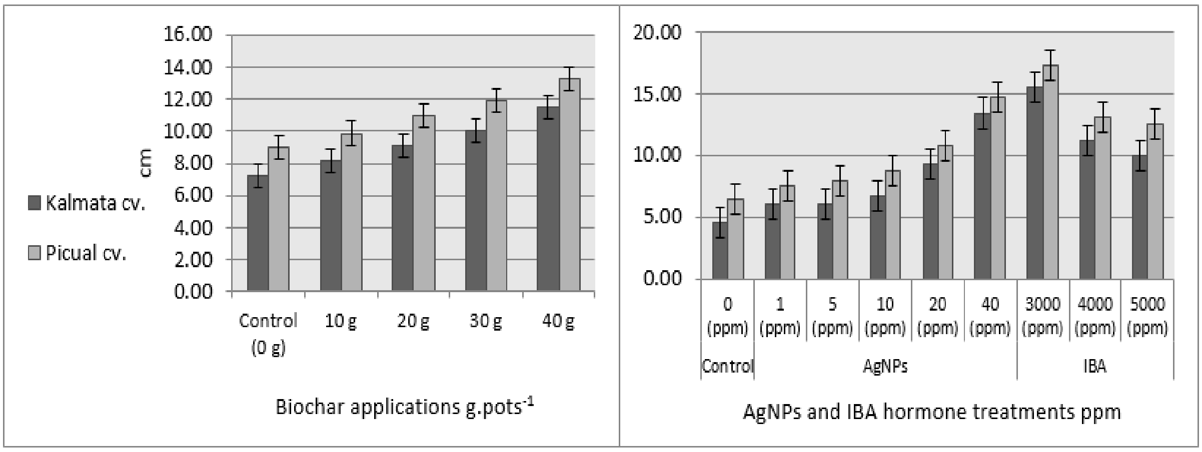

Figure 5. Roots length of Kalamata and Picual olive cultivar cuttings treated by Ag nanoparticles, IBA and biochar applications at different rates. Error bars represent standard errors of triplicates.

Table 5. Branch length (cm) of olive cultivars treated by Ag nanoparticles, IBA and biochar applications at different rates (2018 and 2019 seasons)

Cultivars	Biochar (g/pots)	Control 0	AgNPs (ppm) 1	5	10	20	40	IBA (ppm) 3000	4000	5000	Mean of biochar rates
Season 2018											
Kalmata cv.	**0 g**	16.11n	17.32mn	18.75lm	19.18klm	20.48jk	21.45j	22.71i	18.34lmn	16.38n	**18.97D**
	10 g	17.45mn	22.22i	23.67ghi	25.00fgh	25.46fg	25.70efg	27.51de	19.92jkl	19.30kl	**22.91C**
	20 g	17.63mn	23.55hi	25.41fg	25.86efg	28.26cde	29.56cd	29.44cd	22.11ij	19.49kl	**24.59B**
	30 g	18.33lmn	24.45gh	23.91ghi	26.66def	29.52cd	30.45c	32.09ab	23.88ghi	20.27jk	**25.46AB**
	40 g	19.07klm	25.11fgh	26.56def	27.45de	30.83bc	31.06abc	34.33a	26.27ef	21.29j	**26.88A**
Mean of AgNPs and IBA		**17.72G**	**22.53E**	**23.58D**	**24.83C**	**26.91B**	**27.64AB**	**29.22A**	**22.10E**	**19.35F**	
Picual cv.	**0 g**	15.17r	18.16p	19.81no	20.23mn	22.84k	22.03kl	23.11jk	19.59nop	17.09q	**19.78D**
	10 g	18.63op	19.73no	21.43lm	23.93ij	26.62g	27.33f	28.47ef	22.81k	20.78m	**23.30C**
	20 g	18.99op	19.93no	23.79ijk	26.56g	29.54def	30.34de	31.60d	25.33hi	25.14hi	**25.69BC**
	30 g	19.47nop	20.33mn	24.74i	28.68ef	30.13de	31.25d	34.12b	27.35f	26.14gh	**26.91B**
	40 g	20.04n	21.75klm	25.73ghi	29.26def	31.34d	33.44c	37.54a	30.09de	26.40gh	**28.40A**
Mean of AgNPs and IBA		**18.84G**	**19.98F**	**23.10D**	**25.73C**	**28.09B**	**28.88B**	**30.97A**	**25.03C**	**22.73E**	
Season 2019											
Kalmata cv.	**0 g**	14.54o	16.66m	17.38lm	18.52kl	19.44jk	22.11i	23.47gh	19.45jk	15.41n	**18.73D**
	10 g	17.34lm	19.77jk	20.96ijk	23.00ghi	24.97fg	26.96e	28.84cd	25.66ef	16.11mn	**22.34C**
	20 g	18.23klm	20.96ijk	21.67ij	23.79fgh	25.82ef	27.44de	29.70bcd	28.23cde	16.14mn	**23.55B**
	30 g	18.94kl	21.76ij	22.66hi	24.53fg	28.13cde	30.96bc	33.78ab	30.49bc	20.66ijk	**25.55AB**
	40 g	19.23jkl	22.35hi	24.90fg	25.25efg	30.44bc	31.98abc	34.01a	31.79abc	20.87ijk	**26.65A**
Mean of AgNPs and IBA		**17.83G**	**20.30F**	**21.51E**	**23.02D**	**25.76C**	**27.69B**	**29.56A**	**26.92BC**	**17.67G**	
Picual cv.	**0 g**	16.11n	16.16n	17.83lm	18.61kl	19.56jk	20.91ij	24.08gh	20.11j	17.32m	**18.97E**
	10 g	17.62lm	17.56lm	19.29jk	22.01hi	21.15hij	23.88h	29.79de	25.43g	22.22hi	**22.11D**
	20 g	17.96klm	17.74lm	21.41hij	24.43gh	25.13g	26.64fg	32.66bc	28.22e	26.88efg	**24.57C**
	30 g	18.45kl	18.09klm	22.27hi	26.39fg	27.74ef	31.56c	33.73b	30.48d	27.96ef	**26.30B**
	40 g	19.11k	19.36jk	23.16h	26.92efg	29.97de	33.77b	35.08a	30.79d	28.24e	**27.38A**
Mean of AgNPs and IBA		**17.85F**	**17.78F**	**20.79E**	**23.67D**	**24.71C**	**27.35B**	**31.07A**	**27.01B**	**24.52C**	

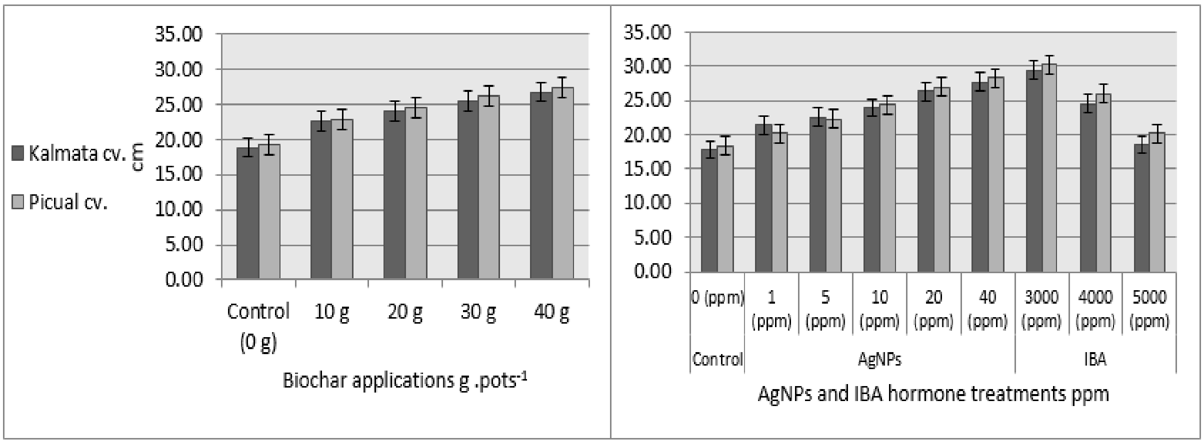

Figure 6. Branch length of Kalamata and Picual olive cultivar cuttings treated by Ag nanoparticles, IBA and biochar applications at different rates. Error bars represent standard errors of triplicates.

Root fresh weights (g)

Data in **Table (6) and Figure (7)** revealed that biochar (BC) caused a high significant increase in root fresh weight (g). The biochar (BC) at 40 g per pot treatment gave the highest values. While, untreated cuttings (control) had the least values in this respect for Kalamata and Picual olive *cvs.* in both seasons. These results go in line with those reported by **Amendola *et al.*, (2017)** were they conclude that the increase of fine root biomass is mainly due to biochar-treated plants and radial growth and occurs during the water shortage period, supporting fruit setting and ripening in grapevine plants. Also, **Changxun *et al.* (2016)** concluded that both shoot and root biomass were more than 50% higher in the biochar treatments than in the non-biochar control of *Poncirus trifoliata* seedlings.As for, the specific effect of silver nanoparticles (AgNPs) and IBA hormone treatments on root fresh weight (g), All these finding was also reflected in **Table (6) and Figure (7)** in which it shows that the IBA hormone influenced the root fresh weight (g). Moreover, the IBA hormone at 3000 ppm treatment had highest values for Kalamata and Picual olive *cvs.* in both seasons, respectively. Meanwhile, control treatment produced the least significant effect in this respect. This means both treatments show insignificant effect in root fresh weight of Kalmata and Picual olive cuttings compared with control. This pattern is similar to that reported by **Khajehpour *et al.* (2014)** who reported that reported that the maximum root fresh weights was observed in olive cuttings treated with IBA whereas the minimum one was observed in control.Regarding, the interaction between silver nanoparticles (AgNPs) and IBA hormone treatments and biochar (BC) applications.**Table (6)** revealed that root fresh weight (g) were significantly increased due to adding IBA hormone × biochar treatments. Furthermore, the IBA hormone at 3000 ppm by biochar (BC) at 40 g per pot induced more stimulative effect on root fresh weight (g), while control treatment had the least values in this respect. The other interactions came in between. Picual olive *cv.* was superior on Kalmata *cv.* to root fresh weights (g) per cutting in both seasons.

Generally, high biochar application rates of 40 g per pot were shown to have a positive effect on root parameters of olive cuttings, possibly due to containing organic matter and nutrients **(Table 1)** which increased soil pH, electric conductivity (EC), organic carbon (C), total nitrogen (TN), and available phosphorus (P) **(Dume *et al.*, 2016).** In addition, the biochar amendment to the soil proved to be beneficial to improve soil quality and retain nutrients, thereby enhancing plant growth **(Bonanomi *et al.*, 2017).** The positive influence of biochar with AgNPs or IBA hormone on root ability of olive cutting suggests that using biochar is a good way to overcome nutrient deficiency, making it a suitable technique to improve nursery-scale nutrient cycles. Therefore, a complete focus is been made to explore the positive effects of biochar amendment on soil stability and root cuttings promotion. This study open a new door for investigating the effect of the novel applications of silver nanoparticles and biochar on root abilty of different difficult fruit cuttings taking into account that each fruit species require an individual experimental studies to determine the promising additions of both treatments.

Table 6. Root fresh weight (g) of olive cultivars treated by Ag nanoparticles, IBA and biochar applications at different rates (2018 and 2019 seasons)

Cultivars	Biochar	Control	AgNPs (ppm)				IBA (ppm)			Mean of
	(g/pots)	0	1	5	10	20	40	3000	4000	5000	biochar rates
Season 2018											
Kalmata cv.	**0 g**	5.79j	7.70hi	9.01g	9.46fg	9.84efg	10.45ef	10.33ef	8.88gh	7.28hi	**8.75C**
	10 g	6.60ij	8.78gh	10.54ef	9.93efg	10.23ef	10.76ef	11.88cde	9.86efg	7.65hi	**9.58BC**
	20 g	6.80hij	9.04g	10.86def	10.43ef	11.36de	11.95cde	13.19ab	10.95def	8.49ghi	**10.34B**
	30 g	6.93hij	9.22fg	11.29de	11.06def	12.27bcd	12.91bc	14.24a	11.82cde	10.02efg	**11.08AB**
	40 g	7.42hi	9.87efg	12.08cd	12.16cd	12.39bcd	13.45ab	14.95a	13.00bc	13.12b	**12.05A**
Mean of AgNPs and IBA		**6.71F**	**8.92E**	**10.76BCD**	**10.61CD**	**11.22B**	**11.90AB**	**12.92A**	**10.90BC**	**9.31D**	
Picual cv.	**0 g**	8.13h	8.54gh	9.30fg	10.05f	10.55ef	10.11f	11.07e	9.97f	8.18h	**9.55E**
	10 g	8.59gh	8.88gh	10.14f	10.85ef	11.08e	10.92ef	12.33d	9.47fg	8.86gh	**10.12D**
	20 g	8.67gh	9.14g	11.87de	12.81cd	12.52cd	12.34d	13.69c	8.62	9.66fg	**11.03C**
	30 g	8.93gh	9.60fg	12.46cd	13.96bc	12.90cd	13.45c	14.78b	9.05g	10.43ef	**11.73B**
	40 g	9.83f	10.27ef	13.58c	14.65b	13.54c	14.66b	16.26a	9.69	11.37de	**12.65A**
Mean of AgNPs and IBA		**8.84E**	**9.29D**	**11.47C**	**12.47B**	**12.12B**	**12.29B**	**13.63A**	**9.36D**	**9.69D**	
Season 2019											
Kalmata cv.	**0 g**	6.33n	6.65mn	7.24kl	7.82kl	8.22jk	8.38jk	9.49hi	8.54ijk	7.00lmn	**7.74D**
	10 g	7.22lm	7.81kl	9.48hi	9.14i	10.44fgh	12.12de	12.43d	11.99def	10.56fgh	**10.13C**
	20 g	7.43klm	8.05k	9.77h	9.60h	11.58ef	13.71bc	13.30c	12.11de	10.82fg	**10.71B**
	30 g	7.58klm	8.21jk	10.16gh	10.17gh	12.51d	14.81ab	14.36abc	12.35de	11.04efg	**11.24AB**
	40 g	8.11k	8.78ij	10.87fg	11.19efg	12.64d	15.40a	15.80a	13.59bc	11.15efg	**11.95A**
Mean of AgNPs and IBA		**7.33E**	**7.90E**	**9.51D**	**9.58D**	**11.08BC**	**12.89A**	**13.08A**	**11.72B**	**10.11C**	
Picual cv.	**0 g**	7.71mn	7.90lmn	9.13k	9.98j	10.38ij	10.90hi	9.00k	10.59hij	7.38n	**9.22D**
	10 g	8.79kl	10.25ij	12.00fgh	12.60efg	11.30h	11.90fgh	13.45d	13.24de	9.23jk	**11.42C**
	20 g	9.05k	8.14lm	10.68hij	11.78gh	12.77ef	12.83ef	14.93bc	14.69bcd	11.16hi	**11.78C**
	30 g	9.25jk	8.55klm	11.21h	12.84ef	13.15def	13.72cd	16.12ab	14.99bc	11.61gh	**12.38B**
	40 g	9.88j	9.14jk	12.22fg	13.48d	13.81cd	14.95bc	17.74a	15.58b	12.78ef	**13.29A**
Mean of AgNPs and IBA		**8.93F**	**8.80F**	**11.05D**	**12.14C**	**12.28BC**	**12.86B**	**14.25A**	**13.82AB**	**10.43E**	

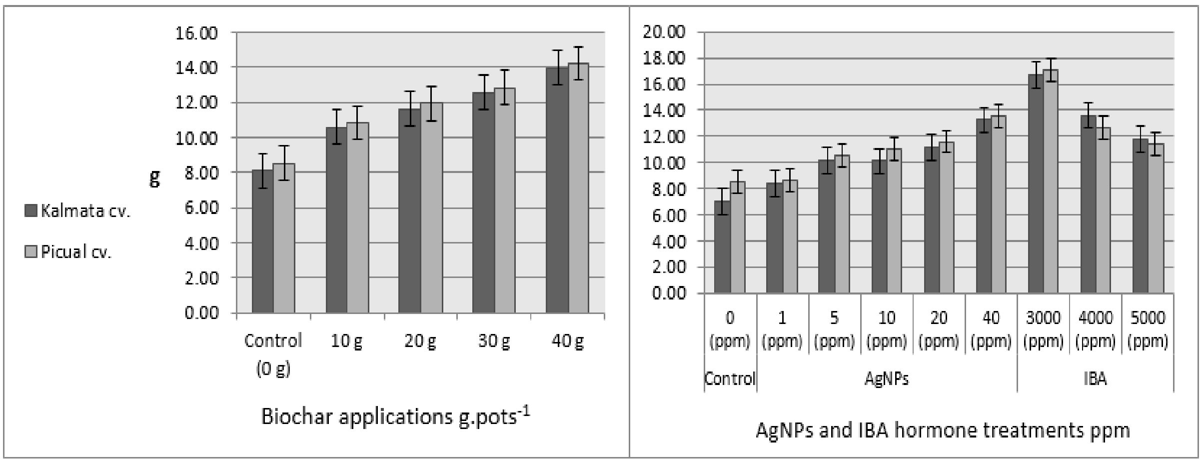

Figure 7. Root fresh weight (g) of Kalamata and Picual olive cultivar cuttings treated by Ag nanoparticles, IBA and biochar applications at different rates. Error bars represent standard errors of triplicates.

CONCLUSIONS

Olive considers one of the economically important fruit crops for agroforestry usage. Therefore, an attention should be been taken into our account to improve its root ability techniques for some economical cultivars using novel methods. Different AgNPs, and olive pomace-derived biochar concentrations were used to increase rooting parameters such as rooted cuttings percentage, number of roots per cutting, root and branch length and root fresh weights of Kalamata and Picual olive cuttings. The results showed significant enhancement on rooted cuttings percentage features compare with the control of both AgNPs and biochar in case of Kalamata and Picual olive cuttings. The results showed that biochar at highest concentration (40 g per pot) had a promising results comparing to other treatments. However, the concentration of 40 ppm of AgNPs as well as IBA hormone at 3000 ppm showed also the highest Kalamata and Picual olive cuttings rooting parameters. The enhancement of Kalamata and Picual olive cuttings as a result of adding biochar could be attributed to the existence of nutrient content in it. The current results should be more investigated under soil condition to figure out the impact of different soil properties on AgNPs and biochar behaviours in contact with olive and cuttings utilizing the current results.

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