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EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI AND BIOCHAR ON EVENING PRIMROSE (*OENOTHERA BIENNIS* L.) PLANT

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ABSTRACT: A field experiment was done to investigate the effect of biochar application (0, 1 and 2 t/fed) and arbuscular mycorrhizal fungi (AMF) inoculation (un-inoculation, seed-inoculation and after-germination inoculation) on growth, yield and quality of evening primrose plants (*Oenothera biennis* L.) during the two seasons of 2021/2022 and 2022/2023 at a private farm in El-Sadat City, El-Menoufia Governorate, Egypt. The results showed that growth parameters, flowering characteristics, carbohydrates content, NPK content, seed yield and fixed oil yield were increased with biochar addition especially at 2 t/fed. The greatest outcomes of AMF inoculation were observed when it was carried out after-germination. Application of biochar by 2 t/fed combined with add AMF after-germination inoculation gave the highest values of growth parameters, flowering characteristics, total carbohydrates content, NPK content, seed yield and fixed oil yield of evening primrose plants. The study concludes that integrating biochar and AMF can significantly enhance the growth (i.e., plant height, number of branches, number of leaves per plant, leaf fresh and dry weights per plant and leaf area), yield (i.e. seed weight per plant and per feddan, fixed oil yield per plant and feddan), and quality of evening primrose, offering a sustainable approach to improving medicinal and aromatic plant production.

Keywords: Evening primrose, *Oenothera biennis* L., biochar, arbuscular mycorrhizal fungi, fixed oil

INTRODUCTION

Evening primrose (*Oenothera biennis* L.) is a notable member of the Onagraceae family, recognized for its distinctive yellow flowers that bloom in the evening. It is native to North America. Because of its ornamental and medicinal characteristics; this biennial plant has spread around the world. It typically growths reach to 1.5 meters in height, with alternately arranged leaves and serrated edges. The plant produces capsule fruits containing numerous seeds rich in gamma-linolenic fatty acid with significant health benefits (Villalobos *et al*., 1998 and Liu *et al*., 2003). For its medicinal properties, evening primrose has long been utilized, especially in the treatment of rheumatoid arthritis, eczema, and premenstrual syndrome. (Anstey *et al*., 2009 and Hajiaghayi1 *et al*., 2024). As a result of recent research highlighting the plant's ability to regulate hormone imbalances and its anti-inflammatory properties, evening primrose fixed oil is highly valued for its high gama-linolenic content, which is known for its anti-inflammatory and immunomodulatory properties, it may help manage diabetic neuropathy by improving nerve function and reducing pain and it is used as a nutritional supplement and in cosmetic products, it is also used as a treatment for rheumatoid arthritis, eczema and premenstrual syndrome and it has been well-documented for reducing the symptoms of atopic dermatitis and mastalgia, making its oil a favored supplement for managing a variety of health conditions. It is used in skincare products to improve the suppleness and hydration of the skin, which helps with issues like acne and aging skin (Anstey *et al*., 2009, Lin *et al*., 2018; Mahboubi, 2019 and Prado and Adiao, 2024).

Biochar (BC), a carbon-rich substance obtained through the thermal breakdown of organic material without oxygen (pyrolysis), has drawn a lot of interest in sustainable agriculture and environmental management (Jeffery *et al*., 2011 and Lehmann and Joseph, 2024). Because of its special qualities, which include high porosity, a big surface area, and the ability to exchange cations, biochar is a great soil supplement that may enhance soil structure, water retention, and nutrient availability (Verheijen *et al*., 2010 and Gao *et al*., 2022). Studies have indicated that the use of biochar can have a notable impact on plant development and output by augmenting soil fertility and microbial activity (Jeffery *et al*., 2011 and Sánchez-Reinoso *et al*., 2020). According to many studies, biochar may lower soil bulk density, which promotes root penetration and plant development, and raise soil pH, which is especially helpful for acidic soils. Furthermore, biochar's capacity to hold onto water and nutrients lessens the need for artificial fertilizers and irrigation, which makes it an affordable and sustainable agricultural option (Al Masud *et al*., 2022 and Lehmann and Joseph, 2024). The synergistic effects of biochar with other soil amendments, such as organic and chemical fertilizers, further amplify these benefits, promoting healthier plant development and higher yields (Abd Wahab *et al*., 2023). Biochar serves as a long-term carbon sink by fixing organic carbon in the soil, which is essential for reducing greenhouse gas emissions (Jeffery *et al.*, 2011). It also aids in the remediation of contaminated soils by adsorbing heavy metals and organic pollutants, reducing their bioavailability and toxicity (Gogoi *et al*., 2021 and Kabir *et al*., 2023). Moreover, biochar's impact on the soil microbial community, including beneficial fungi and bacteria, can enhance soil biodiversity and resilience against plant diseases (Hu *et al*., 2024). Thus, integrating biochar into agricultural practices supports crop production and broader environmental sustainability goals. Biochar has demonstrated significant improvements in the growth, yield, and quality of various medicinal and aromatic plants by improving soil properties such as pH balance, nutrient retention, and microbial activity. For instance, Jabborova *et al*. (2021) found that biochar increased plant biomass and enhanced essential oil concentration in *Ocimum basilicum*. Mumivand *et al*. (2023) reported similar benefits in *Mentha piperita*, attributing improvements to enhanced soil structure and nutrient availability. In another study, Langeroodi *et al*. (2021) examined the impact of biochar on sunflower plant, noting increased root biomass and bioactive compound concentration. Abd Wahab *et al.* (2023) found that combining biochar and compost significantly boosted plant growth and essential oil production in *Lavandula angustifolia*.

Arbuscular mycorrhizal fungi (AMF) are a group of soil-borne fungi that form mutualistic associations with the roots of most terrestrial plants. Arbuscular mycorrhizal fungi enhance plant growth by facilitating the uptake of water and essential nutrients, such as phosphorus, from the soil (Bennett and Groten, 2022). They also help plants to overcome various abiotic stresses, including drought and salinity, by improving soil structure and increasing root surface area (Moghith, 2019 and Parihar *et al*., 2024). Additionally, AMF play a crucial role in soil health by promoting soil microbial diversity and resilience against plant pathogens (Smith and Read, 2008). The symbiotic relationship between AMF and plants is vital for sustainable agriculture, as it reduces the need for chemical fertilizers and enhances crop productivity (Gianinazzi *et al*., 2010 and Johny *et al*., 2021) showed an increased concentration of withanolides bioactive compounds due to AMF application. Assis *et al.* (2020) demonstrated that combining AMF and organic fertilizers significantly boosted the growth and essential oil yield of *Melissa officinalis* L. improving soil health, nutrient availability, and plant resilience. Arbuscular mycorrhizal fungi enhance plant growth, nutrient content, yield and quality (Ramadan, 2019 and Shalaby and Ramadan, 2024). The use of AMF and biochar as soil amendments has shown promise in enhancing plant growth, nutrient uptake, and soil health, offering potential benefits for evening primrose cultivation (Zolfaghari *et al*., 2013; Goudarzian *et al*., 2021 and Zhao *et al*., 2022). This study aimed to examine how the combination of biochar application and AMF inoculation influences the growth, flowering characteristics, nutrient content, seed yield, and oil yield of the evening primrose plant.

MATERIALS AND METHODS

Site description and experimental design:

The research was conducted at a private farm in El-Sadat City, El-Menoufia Governorate, Egypt during 2021/2022 and 2022/2023 seasons. Evening primrose seeds were obtained from Sekem Co., Egypt. The seeds were sown in open field on October 15th of each season directly in hills at a rate of ten seeds per hill, spaced 60 cm apart with 30 cm between hills on one side of each row. Drip irrigation system with drippers (2.0 liter/hour/hill) was utilized throughout the duration of both seasons. Drippers were set up at 2.0 liter/hour/plant for just two hours every two to three days. Three weeks after-sowing, seedlings were thinned to three plants per hill and further thinned to one plant per hill (approximately 23,000 plants per feddan). According to Rainwater and Thatcher (1960) and Black *et al*. (1982), the chemical analyses of the experimental soil and irrigation water are shown in Table (1). The experiment was performed under a split-plot design in three replicates, the main plot was employed by the biochar levels (0, 1 and 2 t/fed), while the subplot was devoted to the AMF inoculation (un-inoculation, seed-inoculation, and after-germination inoculation). The plants were harvested on 30th and 29th April in the first and second seasons, respectively. The vegetative parts were cut approximately 1 cm above the soil surface.

Application of treatments:

Biochar was added to the soil at predetermined rates during the soil preparation stage for sowing. The source of biochar was Miegos Company, Cairo, Egypt. Table (2) presents the chemical analysis of the used biochar.

The inoculum of AMF was obtained from the Agricultural Microbiology Department, National Research Center, Egypt. The inoculum was previously isolated from Egyptian soils and propagated on sterilized peat, vermiculite, and perlite (v/v/v) (Badr El-Din *et al*., 1999). The AMF inoculum consisted of roots, hyphae, spores, and growth media from pot cultures of onion plants colonized with Glomus mosseae NRC31 and *Glomus fasciculatum* NRC15. For seed inoculation, 20 g of inoculum was mixed with 5 g of *O. biennis* seeds before sowing. For after-germination inoculation, 3.5 g of inoculum was mixed with the soil around the seedling roots from four sides after 15 days from sowing. The experiment included 9 treatments with three replicates, each replicate consists of 10 plants i.e. 30 plants in each treatment.

Data collection:

Throughout the growth periods of both seasons, various growth parameters of *Oenothera biennis* were meticulously

Table 1. Chemical analysis of soil and water.

Material	pH	EC dS m^{-1}	K^+ (mM)	Ca^{++} (mM)	Mg^{++} (mM)	Na^+ (mM)	Cl^- (mM)	SO_4^{--} (mM)	HCO_3^- (mM)	CO_3^{--} (mM)
Soil	7.9	2.99	1.05	9.62	6.0	11.5	14.2	10.70	3.2	0.0
Water	7.4	0.53	0.31	3.25	0.25	1.48	1.65	0.72	2.95	0.0

Table 2. Chemical analysis of used biochar.

pH	EC dS m^{-1}	N %	P%	K%	Organic C %	O/C ratio	H/C ratio
8.5	2.52	1.5	7.0	2.0	70.0	0.6	0.36

monitored and recorded. Key parameters included:

Plant growth parameters: plant height (cm), number of branches, number of leaves per plant, leaves' fresh and dry weight per plant (g) and leaf area (cm^2). The leaf disk method was used to estimate the leaf area (Hafez *et al*., 2018). Ten leaf disks were taken using a test tube known for its area (1.76 cm^2). The leaf area was calculated using the following formula:

$$\text{Leaf area} = \frac{\text{Weight of leaves (g/plant)} \times 10n}{\text{Weight of 10 leaf disks (g/plant)}}$$

where n = area of one disk

Flowering characteristics: number of flowers per plant and fresh weight of flowers per plant (g) during all flowering period (Thakur *et al*., 2019 and Ghatas and Mohamed, 2020).

Seeds yield parameters: number of capsules per plant and fresh and dry weight of capsules per plant, seed weight per plant (g) and per feddan (kg), seed fixed oil yield per plant (g) and per feddan (kg).

Chemical constituents: NPK, carbohydrate content, fixed oil percentage, fixed oil yield per plant and per feddan. N % was determined using the modified micro Kjeldahl method as described by A.O.A.C. (1970). P % was measured colorimetrically according to the method described by Murphy and Riley (1962). K % was estimated using flame photometer following the procedure outlined by Cottenie *et al.* (1982). Carbohydrate content in the dried leaves was determined according to Chaplin and Kennedy (1994).

Statistical analysis:

Data from the experimental were subjected to statistical analysis to evaluate the individual and interactive effects of biochar and AMF inoculation methods on the growth and productivity of evening primrose. Data from the studied factors were subjected to analyses of variance (ANOVA) using MSTAT-C statistical software package. The differences between the mean values of various treatments were compared by using the least significant differences (L.S.D.) at 0.05%, by Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

Plant growth parameters:

Data presented in Tables (3 and 4) showed that, biochar application significantly enhanced various growth parameters during the two seasons. The application of biochar by 2 t/fed resulted in the tallest plants and the greatest number of branches, also increased the leaf area and the number of leaves per plant. Correspondingly, the fresh and dry weights of leaves were significantly higher with biochar application (2 t/fed). AMF inoculation positively influenced the plant growth parameters. Plants inoculated with AMF after germination was taller and had more branches, also gave the highest values of leaf area, number of leaves per plant, fresh and dry weights of leaves compared to other AMF treatments. Concerning the interaction between biochar and AMF inoculation in this experiment, it was found that the highest values of plant growth parameters were obtained from the combination of biochar application by 2 t/fed with after-germination AMF inoculation in the two seasons. These results align with existing literature highlighting biochar's ability to improve soil structure, water retention, and nutrient availability, thereby promoting plant growth (Lehmann and Joseph, 2024 and Jeffery *et al*., 2011). The application of biochar alone significantly enhanced various growth parameters of evening primrose plants. The observed increases in plant height, number of branches, leaf area, and leaf biomass with biochar treatment can be attributed to its ability to improve soil physical properties and nutrient retention. Biochar's porous structure increases soil aeration, water retention, and cation exchange capacity, which are crucial for root growth and nutrient uptake (Lehmann and Joseph, 2024). Additionally, biochar has been shown to enhance microbial activity in the soil, further aiding nutrient cycling and availability (Jeffery *et al*., 2011 and Sánchez-Reinoso *et al*., 2020).

Table 3. Effect of biochar and AMF and their interaction treatments on plant height (cm), number of branches and leaf area/plant (cm^2) of evening primrose (*Oenothera biennis* L.) plant during 2021/2022 and 2022/2023 seasons.

Treatments		Plant height (cm)				Number of branches				Leaf area/plant (cm^2)			
		Un. AMF	AMF seed inoc.	AMF after germ.	Mean	Un. AMF	AMF seed inoc.	AMF after germ.	Mean	Un. AMF	AMF seed inoc.	AMF after germ.	Mean
First season													
Without BC		86.3	94.5	98.6	93.2	5.13	5.67	6.67	5.82	121.3	142.3	154.3	139.3
1 ton BC/fed		106.3	114.6	121.2	114.0	7.33	8.67	9.33	8.44	159.3	169.7	175.3	168.1
2 ton BC/fed		111.7	126.0	130.3	122.7	8.00	9.33	10.00	9.11	164.3	181.7	187.3	177.8
Mean		101.4	111.7	116.7		6.82	7.89	8.67		148.3	164.6	172.3	
L.S.D at 0.05	**BC**	4.69				1.74				10.57			
	AMF	7.84				0.76				5.85			
	BC×AMF	13.58				1.32				10.13			
Second season													
Without BC		89.8	93.7	97.5	93.7	4.67	6.00	7.00	5.89	123.9	134.5	159.0	139.1
1 ton BC/fed		95.7	119.8	125.9	113.8	8.00	9.33	10.00	9.11	161.0	176.0	179.7	172.2
2 ton BC/fed		107.5	129.9	138.7	125.4	8.33	11.33	11.67	10.44	166.0	184.7	192.7	181.1
Mean		97.7	114.4	120.7		7.00	8.89	9.56		150.3	165.1	177.1	
L.S.D at 0.05	**BC**	4.33				0.50				11.48			
	AMF	4.77				1.26				7.49			
	BC×AMF	8.27				2.18				12.97			

AMF: arbuscular mycorrhizal fungi, BC: Biochar, Un: Uninoculation, Inoc: inoculation, germ: germination

Table 4. Effect of biochar and AMF and their interaction treatments on number of leaves/plant, fresh leaves/plant (g) and dry leaves/plant (g) of evening primrose (*Oenothera biennis* L.) plant during 2021/2022 and 2022/2023 seasons.

Treatments		Number leaves/plant				Fresh leaves/plant (g)				Dry leaves/plant (g)			
		Un. AMF	AMF seed inoc.	AMF after germ.	Mean	Un. AMF	AMF seed inoc.	AMF after germ.	Mean	Un. AMF	AMF seed inoc.	AMF after germ.	Mean
First season													
Without BC		92.67	98.7	102.3	97. 9	103.7	110.3	115.3	109.8	15.83	18.50	21.60	18.64
1 ton BC/fed		107.7	120.0	125.7	117.8	123.0	135.0	142.3	133.4	24.67	28.10	33.10	28.62
2 ton BC/fed		115.3	136.3	145.3	132.3	125.7	148.7	157.7	144.0	26.53	37.17	39.63	34.44
Mean		105.2	118.	124.4		117.4	131.3	138.4		22.34	27.92	31.44	
L.S.D at 0.05	**BC**	11.17				8.11				3.01			
	AMF	4.60				3.34				3.20			
	BC×AMF	7.96				5.76				5.54			
Second season													
Without BC		94.0	99.5	103.7	99.1	107.9	115.7	124.7	116.1	15.63	21.17	23.63	20.14
1 ton BC/fed		109.9	122.6	126.3	119.6	125.3	140.0	147.0	137.4	23.07	29.87	34.19	29.04
2 ton BC/fed		117.6	142.3	152.3	137.4	132.0	159.0	165.7	152.2	26.64	39.02	41.87	35.84
Mean		107.2	121.5	127.4		121.7	138.2	145.8		21.78	30.02	33.23	
L.S.D at 0.05	**BC**	7.67				9.89				1.10			
	AMF	4.01				3.09				2.52			
	BC×AMF	6.94				5.35				4.37			

AMF: arbuscular mycorrhizal fungi, BC: Biochar, Un: Uninoculation, Inoc: inoculation, germ: germination

These findings also support the role of AMF in improving nutrient uptake, soil structure, and plant resilience against abiotic stresses, contributing to better plant growth and biomass (Smith and Read, 2008 and Parihar *et al*., 2024). Inoculation with AMF, particularly after-germination, significantly improved plant growth parameters. AMF form symbiotic relationships with plant roots, enhancing the absorption of essential nutrients like phosphorus, which is vital for plant development and stress resistance (Smith and Read, 2008). The observed increase in plant height, number of branches, leaf area, and biomass in AMF-treated plants is consistent with previous studies that have highlighted the role of AMF in improving plant growth and resilience to abiotic stresses (Gao *et al*., 2022 and Parihar *et al*., 2024). The improvement in plant growth due to AMF can also be attributed to better root architecture and increased surface area for nutrient uptake (Berruti *et al*., 2016). This synergistic effect underscores the potential of integrating biochar and AMF to maximize vegetative growth and overall plant health in evening primrose (Verheijen *et al*., 2010 and Mohammadi *et al*., 2019). The combination of biochar and AMF resulted in the most significant improvements in plant growth parameters, indicating a synergistic effect. This interaction is likely due to biochar improving the soil environment, making it more conducive for AMF colonization and activity. Biochar provides a habitat for AMF spores and hyphae, enhancing their survival and function in the soil (Warnock *et al*., 2007). The combined use of biochar and AMF maximizes nutrient availability and uptake while improving soil structure and health, leading to better overall plant growth and productivity (Verheijen *et al*., 2010 and Mohammadi *et al*., 2019).

Flowering characteristics:

The findings from Table (5) highlight the significant impact of biochar and AMF on the reproductive performance of evening primrose (*Oenothera biennis* L.), particularly in terms of the number of flowers per plant

Table 5. Effect of biochar and AMF and their interaction treatments on number of flower/plant and fresh weight of flower/plant (g) of evening primrose (*Oenothera biennis* L.) plant during 2021/2022 and 2022/2023 seasons.

Treatments		Number of flower/plant				Fresh weight of flower/plant (g)			
		Un. AMF	AMF seed inoc.	AMF after germ.	Mean	Un. AMF	AMF seed inoc.	AMF after germ.	Mean
First season									
Without BC		119.3	127.3	134.3	127.0	11.67	16.50	21.20	16.46
1 ton BC/fed		137.3	154.3	162.0	151.2	25.50	38.60	42.60	35.57
2 ton BC/fed		141.7	181.7	194.7	172.7	31.57	44.90	48.83	41.77
Mean		132.8	154.4	163.7		22.91	33.33	37.54	
L.S.D at 0.05	BC		4.76				3.54		
	AMF		7.90				2.18		
	BC×AMF		13.69				3.77		
Second season									
Without BC		114.7	125.3	136.0	125.3	11.37	15.43	22.90	16.57
1 ton BC/fed		135.0	157.0	166.3	152.8	26.02	39.23	44.60	36.62
2 ton BC/fed		139.3	173.7	192.3	168.4	30.57	47.61	51.04	43.07
Mean		129.7	152.0	164.9		22.65	34.09	39.51	
L.S.D at 0.05	BC		7.95				1.37		
	AMF		7.74				2.92		
	BC×AMF		13.40				5.06		

AMF: arbuscular mycorrhizal fungi, BC: Biochar, Un: Uninoculation, Inoc: inoculation, germ: germination

and the fresh weight of flowers per plant. Both biochar and AMF, whether applied individually or in combination, demonstrate substantial benefits for flower production, which has critical implications for cultivating evening primrose as a medicinal and ornamental plant. Biochar application alone significantly enhanced the floral characteristics of evening primrose plants, the highest biochar level (2 t/fed) resulted in the greatest number of flowers (194.70 and 192.30) and the highest fresh weight of flowers per plant (48.83 and 51.04) in the first and second seasons, respectively.

AMF inoculation, particularly after-germination, also had a significant positive impact on the floral characteristics of evening primrose. The highest values of a number of flower/plant (163.70 and 164.90) and fresh weight of flower/plant (37.54 and 39.51) were obtained from AMF inoculation after germination, in the first and second seasons respectively. The interaction of biochar and AMF exhibited a significant effect, resulting in the highest floral metrics in both seasons. The interaction treatment of 2 ton biochar per feddan with AMF inoculation after-germination resulted in the highest values of number of flower/plant (194.70 and 192.30) and fresh weight of flower/plant (48.83 and 51.04) in the first and second seasons, respectively. These improvements can be attributed to biochar's ability to enhance soil properties, including increased nutrient retention, improved soil structure, and enhanced water-holding capacity. Such improvements in soil conditions promote healthier plant growth and greater reproductive success (Lehmann and Joseph, 2024). The beneficial effects of AMF can be linked to their role in improving nutrient uptake, especially phosphorus, which is crucial for flower development. AMF enhance root growth and increases the root surface area, facilitating better water and nutrient absorption, which in turn supports greater flower production (Smith and Read, 2008 and Parihar *et al*., 2024). This synergistic effect can be attributed to the complementary mechanisms of biochar and AMF. Biochar improves soil structure and nutrient availability, creating a favorable environment for AMF colonization. In turn, AMF enhance nutrient uptake efficiency, leading to improved plant growth and higher flower yields. The interaction between biochar and AMF thus maximizes the reproductive potential of evening primrose (Verheijen *et al*., 2010 and Mohammadi *et al*., 2019).

Seeds yield parameters:

The results in Tables (6 and 7) highlight the significant effects of biochar and AMF on the reproductive and seed yield parameters of evening primrose (*Oenothera biennis* L.). The application of biochar significantly enhanced the number of capsules per plant, as well as the fresh and dry weights of capsules in both seasons. Application of biochar by 2 t/fed gave the highest values of number of capsules, fresh and dry weights in both seasons. Biochar also substantially increased seed weight per plant and per feddan, the maximum values of seed weight per feddan were 974.4 and 1039.1 kg/fed) in the first and second seasons, respectively. AMF inoculation, particularly after-germination, positively impacted the reproductive parameters by increasing the number of capsules, flower fresh weight, flower dry weight, plant seed weight and seed weight per feddan in both seasons. The AMF inoculation after germination treatment gave the highest values of seed weight per feddan was 772.9 kg and 790.9 kg/fed in the first and second season, respectively. The interaction between biochar and AMF inoculation was significant in both seasons. The highest values in all measured parameters were obtained from application of biochar by 2 t/fed with AMF inoculation after-germination. These improvements can be attributed to biochar's ability to enhance soil structure, nutrient retention, and water-holding capacity, creating a more favorable environment for plant growth (Jeffery *et al*., 2011 and Lehmann and Joseph, 2024). Similarly, the benefits observed with AMF are due to its role in enhancing nutrient uptake, particularly

Table 6. Effect of biochar and AMF and their interaction treatments on number of capsules/plant, fresh weight of capsules/plant (g) and dry weight of capsules/plant (g) of evening primrose (*Oenothera biennis* L.) plant during 2021/2022 and 2022/2023 seasons.

Treatments		Number of capsules/plant				Fresh weight of capsules/plant (g)				Dry weight of capsules/plant (g)			
		Un. AMF	AMF seed inoc.	AMF after germ.	Mean	Un. AMF	AMF seed inoc.	AMF after germ.	Mean	Un. AMF	AMF seed inoc.	AMF after germ.	Mean
						First season							
Without BC		193.7	233.0	250.7	225.8	68.67	79.00	86.67	78.11	22.67	27.10	31.07	26.94
1 ton BC/fed		272.3	290.7	307.0	290.0	98.00	123.3	139.71	120.33	34.17	43.17	46.33	41.22
2 ton BC/fed		281.0	329.7	343.0	317.9	111.72	149.70	157.7	139.71	38.00	51.23	58.80	49.34
Mean		249.0	284.1	300.2		92.78	117.30	128.03		31.61	40.50	45.40	
L.S.D at 0.05	BC	14.29				17.79				6.40			
	AMF	9.93				5.90				2.57			
	BC×AMF	17.20				10.23				4.46			
						Second season							
Without BC		210.7	235.7	245.0	230.4	69.30	78.67	85.87	77.95	23.33	28.57	30.80	27.57
1 ton BC/fed		275.0	295.7	300.0	290.2	96.33	122.40	138.04	119.00	33.77	41.40	47.00	40.72
2 ton BC/fed		286.7	322.3	349.7	319.6	107.90	151.20	160.60	139.90	36.80	52.25	61.13	50.06
Mean		257.4	284.6	298.2		91.19	117.40	128.30		31.30	40.74	46.31	
L.S.D at 0.05	BC	23.25				11.25				5.29			
	AMF	19.21				6.58				1.8			
	BC×AMF	33.27				11.39				3.12			

AMF: arbuscular mycorrhizal fungi, BC: Biochar, Un: Uninoculation, Inoc: inoculation, germ: germination

Table 7. Effect of biochar and AMF and their interaction treatments on seeds weight/plant (g) and seeds weight/fed (kg) of evening primrose (*Oenothera biennis* L.) plant during 2021/2022 and 2022/2023 seasons.

Treatments		Seeds weight/plant (g)				Seeds weight/fed (kg)			
		Un. AMF	AMF seed inoc.	AMF after germ.	Mean	Un. AMF	AMF seed inoc.	AMF after germ.	Mean
						First season			
Without BC		15.53	19.43	21.63	18.87	357.3	447.0	497.6	433.9
1 ton BC/fed		24.97	33.17	36.82	31.65	574.2	762.8	846.8	728.0
2 ton BC/fed		29.50	38.87	42.37	36.91	678.5	893.9	974.4	849.0
Mean		23.33	30.49	33.61		536.7	701.2	772.9	
L.S.D at 0.05	BC	3.38				77.77			
	AMF	1.69				38.94			
	BC×AMF	2.93				67.44			
						Second season			
Without BC		15.88	20.13	22.17	19.39	365.2	463.1	449.2	425.8
1 ton BC/fed		25.43	35.30	38.47	33.07	585.0	811.9	884.7	760.5
2 ton BC/fed		31.69	41.47	45.17	39.44	728.9	953.7	1039.1	907.2
Mean		24.33	32.30	35.27		559.7	742.9	790.9	
L.S.D at 0.05	BC	2.84				41.53			
	AMF	1.85				53.55			
	BC×AMF	3.20				92.74			

AMF: arbuscular mycorrhizal fungi, BC: Biochar, Un: Uninoculation, Inoc: inoculation, germ: germination

phosphorus, by improving root architecture and increasing the root surface area (Smith and Read, 2008; Berruti *et al*., 2016 and Parihar *et al*., 2024). The synergy between biochar and AMF likely results from biochar's enhancement of the soil environment, which supports more effective AMF colonization and activity, ultimately boosting nutrient uptake efficiency and plant growth (Verheijen *et al*., 2010; Mohammadi *et al*., 2019 and Gao *et al*., 2022).

Chemical constituents:

The results from Tables (8, 9 and 10) highlight the significant effects of biochar and AMF on the nutrient content, fixed oil percentage, carbohydrate content, and fixed oil yield of evening primrose (*Oenothera biennis* L.). Both biochar and AMF, individually and in combination, demonstrated substantial benefits for improving these critical parameters, which are essential for the medicinal and commercial value of the plant.

Biochar application significantly enhanced the nutrient content of plants. Addition of biochar by 2 t/fed gave the greatest increases in NPK content. The fixed oil yield per plant and feddan also saw substantial increases, indicating that biochar effectively boosts the overall productivity and quality of evening primrose. AMF inoculation, particularly inoculation after-germination significantly improved the nutrient content, fixed oil percentage, carbohydrate content, and fixed oil yield of plant. This improved nutrient absorption results in higher nutrient contents in the plant tissues. Additionally, AMF treatment led to significant increases in fixed oil percentage and carbohydrate content, reflecting enhanced metabolic efficiency and secondary metabolite synthesis.

The interaction of biochar and AMF exhibited a synergistic effect, resulting in the highest values for nutrient content, fixed oil percentage, carbohydrate content, and fixed

Table 8. Effect of biochar and AMF and their interaction treatments on N, P and K content of evening primrose (*Oenothera biennis* L.) plant during 2021/2022 and 2022/2023 seasons.

Treatments		N %				P %				K %			
		Un. AMF	AMF seed inoc.	AMF after germ.	Mean	Un. AMF	AMF seed inoc.	AMF after germ.	Mean	Un. AMF	AMF seed inoc.	AMF after germ.	Mean
First season													
Without BC		2.05	2.11	2.13	2.10	0.217	0.242	0.255	0.238	1.44	1.52	1.73	1.56
1 ton BC/fed		2.19	2.42	2.55	2.39	0.264	0.285	0.312	0.287	1.85	2.02	2.26	2.04
2 ton BC/fed		2.23	2.71	2.79	2.58	0.272	0.235	0.339	0.312	1.93	2.27	2.40	2.20
Mean		2.16	2.41	2.49		0.251	0.284	0.302		1.74	1.93	2.13	
L.S.D at 0.05	**BC**	0.10				0.001				0.20			
	AMF	0.09				0.001				0.13			
	BC×AMF	0.15				0.002				0.23			
Second season													
Without BC		1.99	2.14	2.21	2.11	0.235	0.254	0.275	0.255	1.50	1.56	1.75	1.60
1 ton BC/fed		2.24	2.54	2.67	2.48	0.283	0.300	0.334	0.301	1.92	2.16	2.39	2.16
2 ton BC/fed		2.36	2.84	2.99	2.73	0.299	0.355	0.387	0.347	2.02	2.45	2.59	2.35
Mean		2.19	2.51	2.62		0.272	0.303	0.332		1.81	2.06	2.24	
L.S.D at 0.05	**BC**	0.12				0.004				0.17			
	AMF	0.09				0.003				0.17			
	BC×AMF	0.15				0.005				0.30			

AMF: arbuscular mycorrhizal fungi, BC: Biochar, Un: Uninoculation, Inoc: inoculation, germ: germination

Table 9. Effect of biochar and AMF and their interaction treatments on fixed oil % and carbohydrate content of evening primrose (*Oenothera biennis* L.) plant during 2021/2022 and 2022/2023 seasons.

Treatments		Fixed oil % Un. AMF	Fixed oil % AMF seed inoc.	Fixed oil % AMF after germ.	Fixed oil % Mean	Carbohydrate content Un. AMF	Carbohydrate content AMF seed inoc.	Carbohydrate content AMF after germ.	Carbohydrate content Mean
First season									
Without BC		17.38	17.47	20.42	18.76	13.03	13.98	15.30	14.10
1 ton BC/fed		21.97	22.88	24.22	23.02	15.70	18.34	20.19	18.08
2 ton BC/fed		22.11	25.15	26.28	24.51	16.25	21.28	22.57	20.03
Mean		20.46	22.16	23.64		15.00	17.86	19.35	
L.S.D at 0.05	**BC**		1.97				0.72		
	AMF		1.25				0.54		
	BC×AMF		2.17				0.94		
Second season									
Without BC		18.08	19.10	22.37	19.85	12.89	13.49	15.50	13.96
1 ton BC/fed		22.87	23.03	25.33	23.7	16.05	18.47	21.35	18.62
2 ton BC/fed		23.21	25.50	27.20	25.30	16.55	21.96	22.82	20.44
Mean		21.39	22.54	24.97		15.16	17.97	19.89	
L.S.D at 0.05	**BC**		1.82				0.66		
	AMF		1.60				0.62		
	BC×AMF		2.77				1.07		

AMF: arbuscular mycorrhizal fungi, BC: Biochar, Un: Uninoculation, Inoc: inoculation, germ: germination

Table 10. Effect of biochar and AMF and their interaction treatments on fixed oil yield/plant (g) and fixed oil yield/fed. (kg) of evening primrose (*Oenothera biennis* L.) plant during 2021/2022 and 2022/2023 seasons.

Treatments		Fixed oil yield/plant (g) Un. AMF	Fixed oil yield/plant (g) AMF seed inoc.	Fixed oil yield/plant (g) AMF after germ.	Fixed oil yield/plant (g) Mean	Fixed oil yield/fed (kg) Un. AMF	Fixed oil yield/fed (kg) AMF seed inoc.	Fixed oil yield/fed (kg) AMF after germ.	Fixed oil yield/fed (kg) Mean
First season									
Without BC		2.71	3.57	4.41	3.56	62.3	82.1	101.5	82.0
1 ton BC/fed		5.47	7.62	8.91	7.33	125.1	175.1	205.0	168.6
2 ton BC/fed		6.54	9.80	11.13	9.16	150.4	225.4	255.9	210.6
Mean		4.91	6.99	8.15		112.8	160.9	187.5	
L.S.D at 0.05	**BC**		0.99				22.84		
	AMF		0.63				14.55		
	BC×AMF		1.01				25.20		
Second season									
Without BC		2.87	3.84	4.96	3.89	66.0	88.3	114.0	89.50
1 ton BC/fed		5.81	8.17	9.74	7.90	133.5	187.8	224.0	181.8
2 ton BC/fed		7.37	10.61	12.29	10.09	169.5	244.1	282.8	232.1
Mean		5.35	7.54	8.99		123.0	173.4	206.9	
L.S.D at 0.05	**BC**		1.14				26.23		
	AMF		0.86				19.78		
	BC×AMF		1.49				34.25		

AMF: arbuscular mycorrhizal fungi, BC: Biochar, Un: Uninoculation, Inoc: inoculation, germ: germination

oil yield. The integrated treatment of biochar by 2 t/fed with AMF inoculation after-germination led to the most significant increases in NPK levels, as well as in the fixed oil and carbohydrate content in both seasons. These enhancements are likely due to biochar’s ability to improve soil nutrient retention and availability, as well as its positive effects on soil structure and water-holding capacity (Lehmann and Joseph, 2024). The improved nutrient content translates into higher fixed oil percentages and carbohydrate content in the plant. The findings are supported by studies showing biochar’s role in enhancing soil fertility and plant metabolic functions (Jeffery *et al*., 2011 and Sánchez-Reinoso *et al*., 2020). Biochar improves soil structure, nutrient retention, and water-holding capacity, which creates a more favorable environment for plant growth (Lehmann and Joseph, 2024). Additionally, biochar's ability to sequester carbon and reduce greenhouse gas emissions makes it a valuable tool in promoting environmental sustainability (Gianinazzi *et al*., 2010 and Jeffery *et al*., 2011). AMF are known to enhance nutrient uptake, especially phosphorus, by improving root architecture and increasing the root surface area (Smith and Read, 2008). The fixed oil yield per plant and per feddan was also maximized with AMF treatment, highlighting AMF’s role in boosting overall plant productivity and quality (Gao *et al*., 2022 and Parihar *et al*., 2024). AMF improve nutrient uptake efficiency, leading to better plant growth and higher yields of valuable plant metabolites. Moreover, AMF promote soil biodiversity and resilience against plant pathogens, underscoring their importance in sustainable agriculture (Gianinazzi *et al*., 2010). The synergistic interaction between biochar and AMF can be attributed to biochar’s enhancement of soil conditions, which supports more effective AMF colonization and activity (Verheijen *et al*., 2010 and Mohammadi *et al*., 2019). This combination maximizes the benefits of both amendments, promoting sustainable agricultural practices by improving soil health and reducing reliance on chemical fertilizers. The interaction between biochar and AMF enhances nutrient availability and uptake, leading to improved plant growth and higher yields of valuable plant metabolites (Verheijen *et al*., 2010, Mohammadi *et al*., 2019 and Gao *et al*., 2022). The findings of this study underscore the potential of biochar and AMF as sustainable soil amendments for enhancing the nutrient content, biochemical composition, and oil yield of medicinal plants like evening primrose. Integrating these treatments into agricultural practices can lead to improved plant health, higher yields, and better quality of medicinal products (Gianinazzi *et al*., 2010 and Jeffery *et al*., 2015).

CONCLUSION

In conclusion, the treatment by biochar and/or AMF inoculation has a positive impact on evening primrose (*Oenothera biennis*, L.) quantity and quality. The biochar addition by 2 t/fed and AMF inoculation after germination showed an enhancement in plant growth, seeds yield, chemical constituents and fixed oil productivity of evening primrose plant.

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تأثير الميكروريزا والبيوشار على نبات زهرة الربيع المسائية (*Oenothera biennis* L.)

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تم إجراء تجربة حقلية لدراسة تأثير استخدام البيوشار(الفحم الحيوي) بمعدل (٠، ١ و ٢ طن/فدان) والتلقيح بفطر الميكروريزا (بدون تلقيح، تلقيح البذور وتلقيح ما بعد الإنبات) على نمو وإنتاجية وجودة نباتات زهرة الربيع المسائية (*Oenothera biennis* L.) خلال موسمي ٢٠٢٢/٢٠٢١ و٢٠٢٣/٢٠٢٢ في مزرعة خاصة بمدينة السادات، محافظة المنوفية، مصر. أظهرت النتائج أن صفات النمو، خصائص التزهير، محتوى الكربوهيدرات، النتروجين، الفسفور، البوتاسيوم، محصول البذور ومحصول الزيت الثابت زادت جميعها مع إضافة البيوشار خاصة بمعدل ٢ طن/فدان. لوحظ أن أفضل نتائج التلقيح بالميكروريزا عندما تم إجراؤه بعد الإنبات. أدى إستخدام البيوشار بكمية ٢ طن/فدان مع إضافة الميكروريزا بعد الإنبات إلى الحصول على أعلى قيم لمعايير النمو وخصائص التزهير ومحتوى الكربوهيدرات ومحتوى النتروجين، الفسفور، البوتاسيوم ومحصول البذور ومحصول الزيت الثابت لنباتات زهرة الربيع المسائية. وتستنتج الدراسة أن دمج البيوشار والميكروريزا يمكن أن يعزز بشكل كبير من صفات النمو (إرتفاع النبات، عدد الفروع، عدد الأوراق للنبات، الوزن الطازج والجاف للأوراق للنبات ومساحة الأوراق)، والإنتاجية (محصول البذور ومحصول الزيت الثابت للنبات وللفدان)، وجودة نباتات زهرة الربيع المسائية، مما يقدم نهجاً مستداماً لتحسين إنتاج النباتات الطبية والعطرية.