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The Effects of the Combination of Mycorrhizae, Vermicompost and Humic Acid Applications on Ornamental Sunflower Growth Parameters

Mikoriza, Vermikompost ve Hümik Asit Uygulamalarının Kombinasyonunun Süs Ayçiçeği Büyüme Parametreleri Üzerine Etkileri

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THE EFFECTS OF THE COMBINATION OF MYCORRHIZAE, VERMICOMPOST AND HUMIC ACID APPLICATIONS ON ORNAMENTAL SUNFLOWER GROWTH PARAMETERS

ABSTRACT

This study aimed to investigate the effect of the combination of mycorrhizal fungi, vermicompost, and humic acid application on ornamental sunflower growth. The study employed ornamental sunflower (*Helianthus annuus* L. cv. 'Sunsantion') as the plant material. Seedlings, acquired from a local ornamental production company at the four-true-leaf stage, were planted in plastic pots (2 L) containing peat perlite medium (1:1 v/v). The research incorporated three distinct doses of humic acid (10, 20, and 30 ml/L) and vermicompost (10, 20, and 30 ml/L). Humic acid (HA) and vermicompost (VC) were applied by soil drenching with 200 ml solution per pot. After one week, the seedlings were transferred into the pots. In all Mycorrhizae applications, 2 g/pot mycorrhizae inoculated during seedling transferred into the pots. Additionally, 20 ml of humic acid (HA20/M2) and vermicompost (VC20/M2) were combined with mycorrhiza application and applied to the plants. No application was made to the control group. Relative growth rate (RGR) was calculated from harvested samples of individuals from the same application group at 15 and 30 days after plants were transferred into the pots. The highest RGR obtained from HA20/M2 and vermicompost VC20/M2 treatments was 3.55 and 3.48%, respectively. Additionally, the highest values of flower diameter (11.71 cm), stem length (41.13 cm), stem diameter (8.46 cm), and flower longevity (10.4 days) were observed in VC20/M2 treatment.

Keywords: *Helianthus Annuus*, Organic, Pot Flower, Fertilizer.

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MİKORİZA, VERMİKOMPOST VE HÜMİK ASİT UYGULAMALARININ KOMBİNASYONUNUN SÜS AYÇİÇEĞİ BÜYÜME PARAMETRELERİ ÜZERİNE ETKİLERİ

ÖZ

Bu çalışmada mikoriza, vermikompost ve hümik asit uygulamasının süs ayçiçeği büyümesine etkisinin araştırılması amaçlanmıştır. Çalışmada bitki materyali olarak süs ayçiçeği (*Helianthus annuus* L. cv. 'Sunsantion') kullanıldı. Dört gerçek yapraklı aşamada ticari firmadan alınan fideler, torf:perlit ortamı (1:1 v/v) içeren plastik saksılara (2 L) dikildi. Araştırmada üç farklı dozda hümik asit (10, 20 ve 30 ml/L) ve vermikompost (10, 20 ve 30 ml/L) kullanıldı. Saksı başına 200 ml hümik asit (HA) ve vermikompost (VC) uygulamaları topraktan fidelerin saksıya aktarılmasından bir hafta sonra yapıldı. Mikoriza uygulamalarında 2 g/saksı mikoriza fidelerin saksılara şaşırtılması sırasında aşılandı. Ayrıca mikoriza uygulamasıyla birlikte 20 ml'lik dozlarda hümik asit (HA20/M2) ve vermikompost (VC20/M2) birleştirilerek bitkilere uygulandı. Kontrol grubuna ise herhangi bir uygulama yapılmadı. Nispi büyüme oranı (RGR), bitkilerin saksılara aktarılmasından 15 gün ve 30 gün sonra aynı uygulama grubundaki bireylerin hasat edilen örneklerinden hesaplanmıştır. En yüksek RGR, HA20/M2 ve vermikompost VC20/M2 uygulamalarından sırasıyla %3,55 ve 3,48 olarak elde edilmiştir. Ayrıca çiçek çapı (11,71 cm), gövde uzunluğu (41,13 cm), gövde çapı (8,46 cm) ve çiçek ömrü (10,4 gün) değerlerinde en yüksek değerler VC20/M2 uygulamasında gözlenmiştir.

Anahtar Kelimeler: *Helianthus Annuus*, Organik, Saksılı Süs Bitkisi, Gübre.

1. INTRODUCTION

The sunflower (*Helianthus annuus*) is a member of the Asteraceae family. Historically, it has been utilized as an oil-producing plant, and over the past decade, its appeal for ornamental cultivation has experienced a noteworthy rise (Vital-Vilchis et al., 2020). The sunflower holds a prominent position as a primary choice for cut flowers and garden plants. Its potential as a lucrative potted flowering plant is notable, thanks to short crop time, ease of propagation, and the allure of its attractive flowers (Pallez et al., 2002).

Since the inception of the green revolution, there has been widespread utilization of chemical fertilizers (such as soluble acidic NPK fertilizers), pesticides, and herbicides to boost food productivity in response to the increasing demand for food crops due to population growth. Applying chemical fertilizers contributes to higher crop yields as plants directly or indirectly absorb essential nutrients from these inorganic fertilizers. However, the persistent and extensive use of these chemical fertilizers results in detrimental effects on the agricultural ecosystem, including soil degradation, depletion of crop genetic and microbial diversity, contamination of groundwater, and pollution of the atmosphere (Chaudhry et al., 2009). Furthermore, chemical fertilizers have become indispensable for growers. On the other hand, using organic fertilizer has been proven to contribute to environmental sustainability and agricultural production. Moreover, enhancing soil organic matter through the application of organic fertilization has been identified by researchers to improve the physical and chemical attributes of the soil, and various organic fertilizers could potentially lower input costs (Mete et al., 2023; Şa et al., 2023). Organic fertilization also augments microbial biomass, leading to heightened nitrogen fixation events, ultimately resulting in improved plant yield and quality (Özer and Uzun, 2013; Özdemir and Özer, 2016; Yıldız et al., 2023). Applying farm manure, compost, vermicompost, and beneficial bacteria or fungi to the soil boosts microbial activities, enhancing crop growth. Humic acid plays a pivotal role in soil fertility and plant nutrition, as highlighted by Atiyeh et al. (2002). Soils rich in humic substances support robust plant growth, rendering them more resilient to stress, healthier, and capable of yielding higher quantities of crops with superior nutritional quality (Pettit, 2004).

In ornamental plant production, the beneficial effects of organic substance treatments have been reported in various species. For instance, applying humic acid alone or in combination with other substances increases plant growth in gerbera (Nikbakht et al., 2018), *Tulipa gesneriana* (Ali et al., 2014), *Gladiolus grandiflorus* (Bashir, 2016) and *Antirrhinum majus* (Memon and Khetran, 2014). Additionally, positive effects of humic acid applications on vase life were also reported in cut flowers such as chrysanthemum (Fan et al., 2015), rose (Dastyaran and Farahi, 2015) and gerbera (Ansari et al., 2011). Vermicompost is a natural eco-fertilizer produced by degrading organic matter through the interaction between microorganisms and worms. There is ample scientific evidence that using vermicompost significantly affects plants' growth and productivity. (Durukan et al., 2019). The effects of vermicompost use are well demonstrated in many ornamental plants such as chrysanthemum (Padamanabhan, 2021), gladiolus (Amit et al., 2013), cymbidiums (Hatamzadeh, 2011) and marigold (Tyagi and Kumar, 2006). One of the other effective treatments is mycorrhizal fungi, which are essential soil microorganisms for terrestrial ecosystems and form beneficial symbioses with the root systems of most agricultural plants (Popescu and Popescu, 2022). Xue et al. (2019) reported that mycorrhizae applications improved root surface area and volume and expanded the nutrient absorption area of the root system. Therefore, Kınık and Çelikel (2020) used mycorrhizae to enhance the rooting efficiency of hardwood cuttings in *Rosa canina*. Additionally, Gaur et al. (2000) reported that mycorrhizae treatments promote early flowering, increase flower number, and prologs flowering time in *Petunia hybrida*, *Callistephus chinensis*, and *Impatiens balsamina*.

While ornamental plants aren't intended for consumption, the cultivation process involves widespread use of chemical inputs like pesticides and fertilizers, like other agricultural products. These synthetic substances have adverse effects on both human health and the environment. Organic farming techniques provide viable solutions for cultivating ornamental plants, preserving nature and human well-being, and maintaining market value, just as they do for other agricultural products (Tütüncü, 2022). Therefore, this study aimed to evaluate the effects of vermicompost, mycorrhizae, humic acid, and combining mycorrhizae with humic acid and vermicompost on potted sunflower growth.

2. MATERIAL AND METHOD

2.1. Plant Material

The research was conducted during the summer of 2022 at a polyethylene greenhouse located in the application area of the Agriculture Faculty at Ondokuz Mayıs University, Samsun, Türkiye. Throughout the growing season, hourly measurements of minimum, maximum, and average temperatures were recorded using a data logger within the greenhouse (Figure 1). The study employed ornamental sunflower (*Helianthus annuus* L. cv. 'Sunsantion') as the plant material. Seedlings, acquired from a local ornamental production company at the four-true-leaf stage, were planted in plastic pots (2 L) containing peat perlite medium (1:1 v/v). Irrigation was performed twice weekly with approximately 200 mL of tap water per pot. Notably, no fertilizers or chemicals for pest and disease control were utilized in the study.

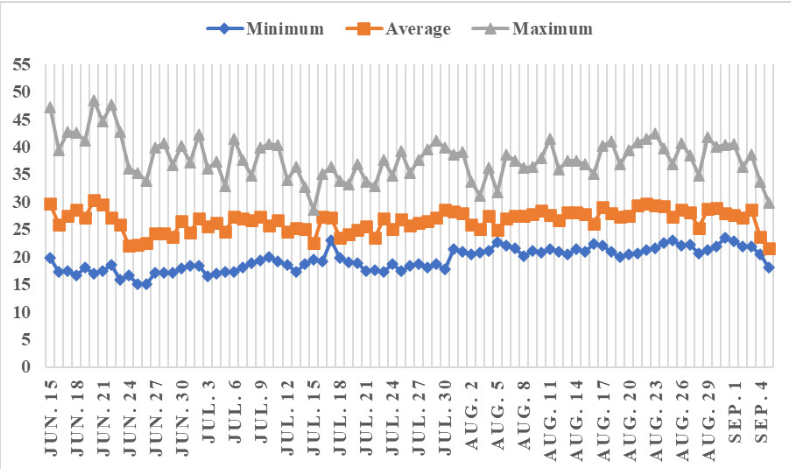

Figure 1. Maximum, minimum, and average temperature (°C) during cultivation in the greenhouse.

2.2. Method

A procedure was followed to acquire the humic acid (HA) utilized. Initially, 310 liters of water were heated to around 80°C. Subsequently, 13 kg of potassium hydroxide was introduced to the heated water. The resulting mixture was then supplemented with 80 kg of leonardite, along with additions of salicylic acid (250 ppm) and IBA (500 ppm), ultimately reaching a total volume of 400 liters (Yıldız et al., 2023). Bacteria-enriched liquid vermicompost (VC) (Orpex Ltd. Türkiye) and 'Endo Roots Soluble Mycorrhiza' (Bioglobal, 2014), consisting of *Gigaspora* *margarita* and eight species of *Glomus* genus mycorrhizal fungi were used as other organic substances. Humic acids and vermicompost treatments were applied to the seedlings once, one week after being transferred to pots. The research incorporated three distinct doses of humic acid (10, 20, and 30 ml/L) and vermicompost (10, 20, and 30 ml/L). HA and VC were applied by soil drenching with a 200 ml solution. For the mycorrhiza application solution, 2 g of mycorrhiza was incorporated into 125 ml of water and applied to the seedlings by soil drenching. Additionally, the concentrations of 20 ml/L of humic acid (HA20/M2) and vermicompost (VC20/M2) were combined with mycorrhiza application. Mycorrhizae are inoculated during seedling transfer into the pots in all mycorrhizae applications. No application was made to the control group (Table 1).

Table 1. Treatments applied in the study.

Applications	Concentrations
Control	Water
Humic acids (HA)	10, 20 and 30 ml/L
Vermicompost (VC)	10, 20 and 30 ml/L
Mycorrhizae	2 g/ 125 ml water
Humic acid + Mycorrhizae (HA20/M2)	20 ml/L + 2 g/ 125 ml water
Vermicompost + Mycorrhizae (VC20/M2)	20 ml/L + 2 g/ 125 ml water

2.3. Evaluated Growth Parameters

The relative growth rate (RGR) was calculated from harvested samples of individuals from the same application group at 15 and 30 days after plants were transferred into the pots. Five plants from each treatment were harvested at first (*t1*) and second harvest time (*t2*), *and samples were dried in an* oven at 70 °C for 48 h. Then, the dry weights of the samples at first (W1) and second harvest time (W2) were measured with an analytical balance. RGR was calculated according to Eq. (1) by Hofmann and Poorter (2002) by modifying the formula as a percentage. $\overline{\ln(W)}$ is the mean of ln-transformed plants dry weight at time (t).

$$RGR = \frac{\overline{\ln(W2)} - \overline{\ln(W1)}}{t2 - t1} \; x\,100 \qquad \text{Eq.}(1)$$

To more clearly reveal the relative growth rates of the applications compared to the control group, the relative growth change (%) was calculated according to Eq. (2). RGC is the mean of relative growth change on the basis of the control group. *RGR (Ai)* is the relative growth rate of application "i," and RGR (A0) is the relative growth rate of the control application.

$$RGC\ (\%) = \frac{RGR(Ai) - RGR\ (A0)}{RGR(A0)}\ x\ 100 \qquad \text{Eq. (2)}$$

Flower longevity of the plants was estimated by observing the flowers from the blooming stage to wilting. At the full bloom stage, stem diameter (cm), stem length (cm), and flower diameter (cm) were determined.

2.4. Experimental Design and Statistical Analysis

The study was arranged following a completely randomized design, featuring ten replications, each involving a single plant. Variance analysis was conducted using JMP statistical software version 8.1, and differences between treatments were assessed using the LSD multiple comparison test.

3. RESULTS AND DISCUSSION

The relative growth rates (RGR) for different treatments were investigated to assess their impact on the overall growth performance of the potted sunflower (Figure 2). According to the results, the control group exhibited a baseline RGR of 2.88%, providing a reference point for evaluating the various treatments.

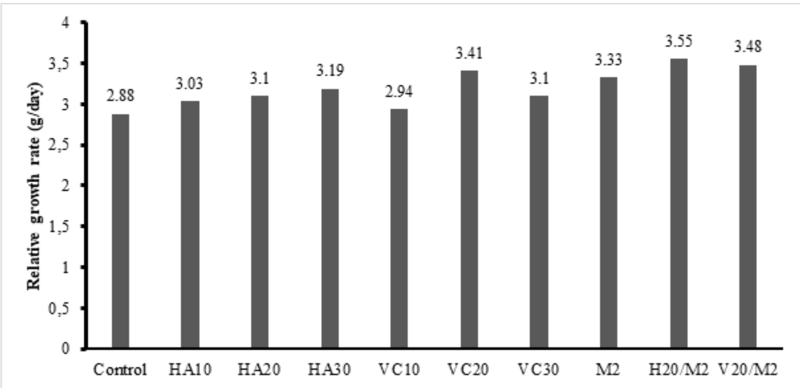

Figure 2. Effects of treatments on relative growth rate.

Among the treatments, the H20 treatment demonstrated a notable increase in RGR, reaching 3.1%, and showcasing a positive effect on the growth rate compared to the control group. Similarly, the H30 treatment exhibited a further enhancement, with a relative growth rate of 3.19%, suggesting a dose-dependent response. The VC10 treatment showed a modest increase in RGR (2.94%), while the VC20 treatment displayed a more substantial improvement, reaching 3.41%. The M2 treatment demonstrated a relatively high RGR of 3.33%, indicating its efficacy in promoting growth. Additionally, the H20/M2 treatment was calculated to be 3.55%, reflecting a synergistic effect when the H20 treatment was combined with the M2 treatment. Similarly, the V20/M2 treatment was estimated to be 3.48, suggesting a positive interaction between the V20 and M2 treatments (Figure 2). When the control application is taken as a reference point for other treatments, H20/M2 and V20/M2 treatments increased relative growth change by 23.2% and 20.8%, respectively (Figure 3). The lowest enhancement in plant growth change was observed in VC10 by 2% and 5.2% in HA10 treatments.

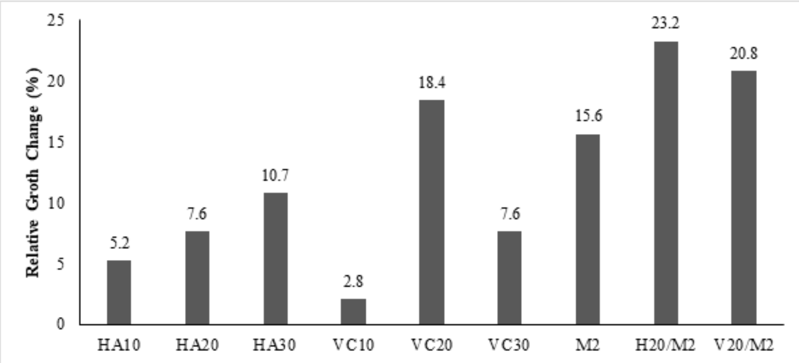

Figure 3. Percentage of relative growth change in the basis of control treatments

Humic acid treatments (HA) at different concentrations affected flower diameters differently. The control group exhibited an average flower diameter of 9.64 cm, the reference point for comparing other treatments. The HA10 treatment resulted in a flower diameter of 9.47 cm, while HA20 slightly increased to 9.68 cm. The highest dose, HA30, significantly enhanced flower diameter to 10.28 cm, indicating a dose-dependent response. Vermicompost treatments (VC) at different concentrations greatly impacted flower diameter. The VC10 treatment resulted in a flower diameter of 10.72 cm, and VC20 maintained a similar level at 10.7 cm. The highest dose, VC30, showed a substantial increase in flower diameter to 11.5 cm, signifying a significant improvement compared to other treatments. The mycorrhizae treatment (M2) displayed a flower diameter of 10.37 cm, indicating a positive effect on growth. When combinations of treatments were considered, the H20/M2 treatment resulted in a flower diameter of 10.72 cm, and the V20/M2 treatment displayed a flower diameter of 11.71 cm (Table 2). These results suggest potential synergistic effects between specific treatments. According to the results, the statistical analysis revealed significant differences between treatments. The highest flower diameter was observed in the VC30 treatment, while the combinations H20/M2 and V20/M2 demonstrated notable effects on flower diameter (Figure 4).

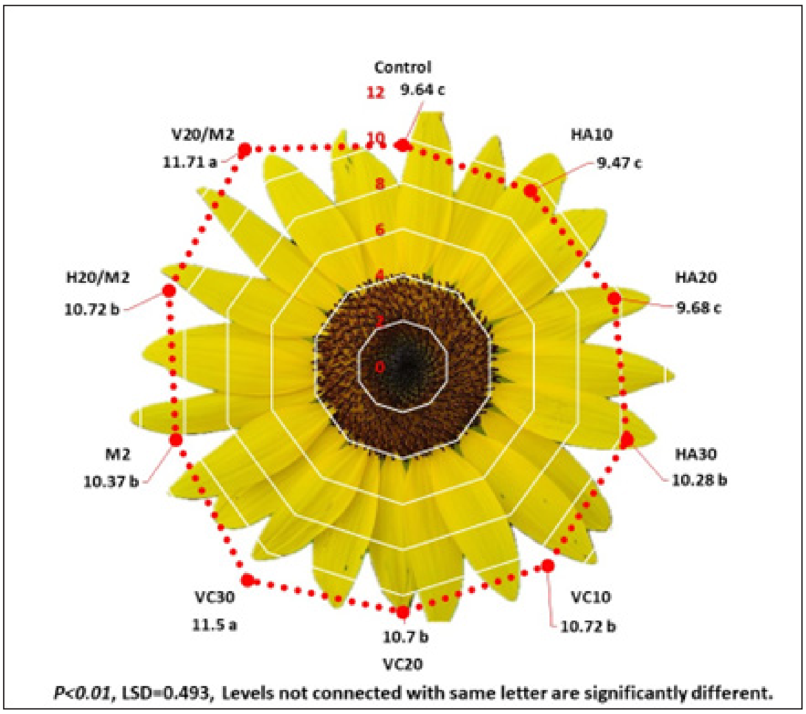

Figure 4. Effects of applications on flower diameter (cm).

Humic acid treatments (HA) at different concentrations displayed a range of stem lengths. The HA20 treatment and control group showed a minimum value of 38.16 cm, while HA30 had a maximum of 40.72 cm among humic acid treatments, indicating a dose-dependent response. Vermicompost treatments (VC) at different concentrations also demonstrated a range of stem lengths. The VC10 treatment had a minimum value of 38.62 cm, while the VC30 treatment exhibited a significant enhancement with a 40.39 cm stem length. The mycorrhizae treatment (M2) resulted in a stem length of 39.74 cm, indicating a positive effect on growth. When combinations of treatments were considered, the V20/M2 treatment exhibited the highest increase in stem length (41.13 cm) among treatments (Table 2).

Table 2. Effects of different organic substances on stem growth and flower longevity.

Treatments (ml/L)	Stem Length (SL) (cm)*	Stem Diameter (SD) (cm)**	Flower Longevity (FL) (days)***
Control	38.16 c	7.83 c	8.6 e
HA10	39.27 abc	8.17 abc	8.93 cde
HA20	38.16 c	8.31 ab	9.4 b-e
HA30	40.72 a	8.20 abc	9.4 b-e
VC10	38.62 bc	7.98 bc	9.86 abc
VC20	39.76 abc	8.07 abc	9.60 a-d
VC30	40.39 ab	7.91 c	9.93 ab
M2	39.74 abc	7.89 c	8.86 de
H20/M2	39.83 abc	7.99 bc	9.86 abc
V20/M2	41.13 a	8.46 a	10.4 a

*$p<0.05$, $LSD_{SL}= 1.99$ **$p<0.05$, $LSD_{SD}= 0.392$; **$p<0.01$, LSDFL= 0.982

The investigation into stem diameter across diverse treatments has provided valuable insights into the potential effects of various amendments on plant growth. The control group exhibited a baseline stem diameter of 7.83 cm, serving as a reference for comparative analysis. HA treatments at different concentrations demonstrated a dose-dependent response, with HA20 yielding the highest diameter of 8.31 cm. Conversely, VC treatments exhibited a more comprehensive pattern with VC20, resulting in a maximum diameter of 8.07 cm. M2 demonstrated a moderate effect with a stem diameter of 7.89 cm. Intriguingly, the combinations of treatments revealed notable synergies, as evidenced by H20/M2 and V20/M2, where V20/M2 produced the highest observed diameter at 8.46 cm (Table 2).

Exploring potted sunflowers' flower longevity under diverse treatments has yielded significant insights. Flower longevity was recorded as the lowest value, with 8.6 days in the control group. Humic Acid (HA) treatments demonstrated a progressive trend with increasing concentrations; HA10, HA20, and HA30 exhibited flower longevity of 8.93 days, 9.4 days, and 9.4 days, respectively. A parallel pattern was observed in Vermicompost (VC) treatments, where VC10, VC20, and VC30 resulted in flower longevity of 9.86 days, 9.60 days, and 9.93 days, respectively. Mycorrhizal treatment (M2) displayed a moderate effect on the flowering period, lasting for 8.86 days. The H20/M2 treatment yielded flower longevity equivalent to VC10, standing at 9.86 days, while V20/M2 showcased the lengthiest duration at 10.4 days (Table 2).

According to the results, humic acid, vermicompost, and mycorrhizae treatments positively affect evaluated parameters in accordance with previous studies.

Mourad et al. (2020) reported that applying humic acid enhances sunflowers' growth, yield, and certain chemical contents cultivated in saline soil conditions. Baldotto and Baldotto (2015) investigated the influence of seed treatment with humic acids on the growth and yield of field-grown ornamental sunflowers (*Helianthus annuus* L.). Multiple variables related to flower stems were assessed during harvest, including height, diameter, weight, leaf count, leaf fresh weight, leaf dry matter, flower stalk count, and floral receptacle diameter, and data revealed consistent increases in most variables, except for the number of leaves per stem, which remained unchanged. Nagarajan and Mahadevan (2002) reported that sunflower plants inoculated with arbuscular mycorrhizal fungi (AMF) showed significant increases in plant growth parameters such as plant height and shoot and root dry weight. Moreover, Gül et al. (2021) found that liquid and solid applications of vermicompost are beneficial to obtaining higher grain and oil yield in oil sunflower. However, it is obvious that combining mycorrhizae with humic acid and vermicompost treatments resulted in better results, which may emphasize potential synergies between specific treatments, while others had varying effects. Additionally, it is well known that humic acid and vermicompost contain nutrients and improve soil nutrient contents. Therefore, the results may suggest that soil enriched with nutrients increases the affinity and activity of mycorrhizae. Hussain et al. (2018) indicated that mycorrhizae application and vermicompost enhanced growth and yield performance in *Triticum aestivum*. Pezeshkpour et al. (2014) reported similar results in chickpea production in another study. Habashy et al. (2008) established a positive correlation between organic waste and compost and the presence of arbuscular mycorrhizal fungi, resulting in significantly higher root infection intensity compared to the control treatment. Previous studies, such as those by Carrenho et al. (2002) and Jan et al. (2014), have also reported increased percentages of root mycorrhizal infection when mycorrhizal inoculums are combined with compost. The elevated infection percentage observed in soil treated with vermicompost likely contributed to enhanced plant nutrition uptake and significantly improved growth and yield parameters.

4. CONCLUSION

In summary, the experimental results suggest that H20M2 and V20M2 treatments positively influence the relative growth rate, stem length, stem diameter, flower diameter, and flower longevity. Additionally, the study may reveal potential synergistic effects between mycorrhizal fungi and vermicompost or humic acid applications. These findings provide valuable insights into the potential applications of these treatments in promoting plant growth and could have implications for various fields, such as agriculture or ecological restoration. However, a more detailed study may be necessary to fully reveal this synergistic effect.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethics

This study does not require ethics committee approval.

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