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Humic acid application on soil stability indices and enzyme activity at different bell pepper water requirements

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Abstract

The use of organic materials helps in reducing the consumption of chemical fertilizers, contributing to the sustainability of agricultural ecosystems. Humic acid can improve soil structure, especially in soils with low clay content, aiding in water retention and enhancing water absorption by plants. This characteristic has led to the attention of humic acid application in drought stress, particularly in arid soil regions. The present study aimed to investigate the simultaneous effects of humic acid and plant water requirements on the physical and biological properties of soil under bell pepper cultivation. The factorial experiment was conducted in a completely randomized block design with three levels of humic acid: 0 (HA_0), 2 (HA_2), and 4 (HA_4) g/Kg of soil and four levels of plant water requirements: 60 (L_{60}), 80 (L_{80}), 100 (L_{100}), and 120% (L_{120}), with four replications under greenhouse conditions. Bell pepper seedlings were transferred to 10 Kg pots after applying humic acid treatments and after the establishment of seedlings, plant water requirement treatments were applied for 80 days. During this period, plants were maintained at a temperature of 25 degrees Celsius during the day, 18 degrees Celsius at night, and a relative humidity of

75%, with a carbon dioxide concentration of 80 milligrams per liter. The results of this study showed that treatments $L_{80}HA_4$, $L_{100}HA_4$, and $L_{120}HA_4$ had the most significant impact on improving soil physical properties, such as normal stability index, total soil stability index, mean weight diameter of soil particles, soil particle stability, geometric mean diameter of soil particles, and reduction in soil particle destruction percentage. The highest values of wet and dry weight diameter of soil particles were observed in treatment $L_{100}HA_4$, which were 42.92%, 74%, and 67.01% higher, respectively, compared to the control ($L_{60}HA_0$). In contrast, the highest percentage of soil particle destruction was observed in treatment $L_{60}HA_0$, showing a 32.90% increase compared to treatment $L_{100}HA_4$. Furthermore, the best performance of bell pepper yield was observed in treatments $L_{80}HA_4$, $L_{100}H_4$, and $L_{120}HA_4$, although the differences between treatments were not statistically significant. The application of humic acid, in addition to improving bell pepper yield, increased water use efficiency and reduced water use. The lowest yield (21.14 g/plant) and water use (3584 m^3/ha) were observed in treatments $L_{60}HA_0$ and $L_{60}HA_4$, respectively. Additionally, the results showed that the highest water use efficiency was observed in the $L_{80}HA_4$ treatment (7.45 Kg/m^3), while the lowest was in the $L_{60}HA_0$ treatment (0.80 Kg/m^3). Moreover, humic acid significantly influenced the activity of soil enzymes, including soil phosphatase and urease. With more application of humic acid, soil moisture retention, microbial biomass carbon, urease, and phosphatase activities increased. However, in treatment with 20 percent more than plant water requirements, enzyme activities decreased.

Keywords: Whole soil stability index, Water use efficiency, Microbial biomass carbon, Phosphatase, and Urease.

Introduction

Soil aggregates are the fundamental units of soil structure and play a crucial role in various physical and chemical processes in the soil, such as soil density, nutrient cycling, soil erosion, root penetration, and crop performance (Cates *et al.*, 2016; Bronick and Lal, 2005). Improved soil structure and greater stability of soil particles are essential for enhancing soil fertility and productivity (Zhang *et al.*, 2016). Soil particle stability is generally determined as a fraction of the remaining particles after exposure to stress-inducing factors (Angers and Carter, 1995) and is considered as a valuable indicator of soil health (Moncada *et al.*, 2015). Indices of soil particle stability include the Whole Soil Stability Index (WSSI), Normalized Stability Index (NSI) and Mean Weight Diameter (MWD) of soil particles, Geometric Mean Diameter (GMD) of soil particles, Aggregate Stability (AS), and Percentage of Aggregate Destruction (PAD) (Li *et al*., 2017).

Organic amendments such as humic acids (HA) play a crucial role in plant growth, agricultural productivity, and soil resilience against non-living stresses. The biological activities resulting from the addition of humic acid to the soil are generally dependent on the amount, origin, molecular size, and water-repellency degree of the humic acid. It directly and indirectly affects plant growth, with improvements in soil physical characteristics, such as density, aeration, permeability, and water-holding capacity, considered indirect effects. Simultaneously, providing more availability of micronutrients is among the direct effects of humic acid (Asik *et al.*, 2009).

Greenhouse development becomes a priority in agricultural production management in arid and semi-arid regions. Particularly, greenhouse vegetable cultivation, which usually requires year-round production, is of great importance. One of the main objectives of greenhouse development in arid and semiarid regions is to improve water use efficiency. The use of water-saving methods in greenhouse cultivation is expected, although a reduction in water use is undoubtedly associated with a decrease in plant performance. Shimono *et al.*, (2007) reported that the highest growth and performance of plants were under non-water stress conditions, and water stress limited plant growth and yield. However, it seems that the use of soil amendments can mitigate the negative effects of reduced irrigation. Zhang *et al.*, (2016) attributed the increase in the Geometric Mean Diameter (GMD) of soil particles to increased organic matter in the soil, which had a positive effect on plant performance. Ali *et al.*, (2019) studied the effect of humic substances and different irrigation levels (100%, 80%, and 60% of standard evapotranspiration) on water use efficiency in potato cultivation. They reported that the highest water use efficiency was observed in the presence of humic substances and 60% of standard evapotranspiration treatments. Ben-gal *et al.*, (2008) also demonstrated in their study the effect of reduced irrigation levels (three levels of 100%, 75%, and 50% of plant water requirements) on bell pepper performance that yield, the number of branches per plant, and the number of fruits decreased with decreasing plant water requirements. Therefore, based on previous studies, the present research was conducted to investigate the effect of humic acid on reducing the negative effects of deficit watering in bell pepper plants under greenhouse conditions.

Materials and Methods

Soil sampling and analysis

This research was conducted in 2022 in the research greenhouse of Payame Noor University in Bojnord. It involved potted cultivation of bell pepper plants for 80 days, from planting to harvest. After plant harvest, soil samples were collected air-dried, and transferred to the laboratory for analysis. Soil texture was determined using the hydrometer method (Gee & Bauder, 1986), and soil electrical conductivity (EC) and pH of saturation extract were determined according to Thomas (1996). Whole-soil stability index (WSSI) and water-stable aggregates (WSA) were determined using the dry elutriation method (Nichols & Toro, 2011). The weights of soil particles in four size classes (0.053-0.25 mm, 0.25-1 mm, 1-2 mm, and 2-5.9 mm) were measured using the dry elutriation method, and the weights of coarse particles (larger than 0.5 mm) were measured using the wet sieving method. Normalized stability index (NSI) was determined for collected soil samples according to Six *et al*. (2000). Soil particle destruction percentage, mean weight diameter (MWD) of particles, geometric mean diameter (GMD) of particles, and particle stability were measured using the wet sieving method and, after correcting for sand content based on Kemper & Rosenau (1986). Water use efficiency (WUE) was calculated using the recommended equation (Zotarelli *et al*., 2009). Soil urease activity was determined using the method by Tabatabai *et al*. (1982). Phosphatase activity (acidic and alkaline) in soil was measured using conventional methods for enzyme activity measurement (Tabatabai *et al*., 1969). The fumigation-extraction method determined microbial biomass carbon in soil (Vance *et al*., 1987). The data obtained from these experiments were analyzed using SPSS version 16, and the means were compared using the Duncan test at a 5% significance level.

Table 1: Physicochemical Properties of Initial Soil in the Experiment

Zn	Fe	K	P	$N_{(T)}$	OC	EC	pH	Texture	Clay	Silt	Sand
mg/kg (ava.)				%		dS/m			%		
0.36	4.60	215	7.35	0.05	1.06	2.48	7.10	Cl	29	49	22

Experimental design and pot preparation

The present study was conducted in the form of a randomized complete block design with factorial arrangement in the research greenhouse of Payame Noor University in Bojnord. The experimental factors included soil moisture content at four levels: L_{60}, L_{80}, L_{100} and L_{120} (60, 80, 100, and 120 % plant requirements, based on soil moisture depletion), and humic acid application at three levels: HA_0 (zero), HA_2 and HA_4 (g/Kg soil). To prepare the pots, the soil was sieved through a 4 mm mesh sieve, and 10 Kg portions were weighed for use in each pot. Then, the amounts of humic acid according to the experimental treatments were added to the weighed soil.

Cultivation of bell pepper and moisture treatments

Treated soil samples saturated with distilled water and gravitational water drainage from the bottom of the pots were monitored at specific time intervals, and approximately 24 hours after the initiation, gravitational water drainage was stopped. The weight of the pots in this condition was considered as the weight at field capacity (Pourmansour *et al*., 2019). After cultivating bell pepper seedlings in the greenhouse, seedlings were transferred to the 10 kg pots with their moisture at field capacity. In each pot, two bell pepper seedlings with an aerial shoot length of 8 cm were planted. Moisture treatments were applied after 15 days of transplanting, and the soil moisture content was adjusted based on treatments every 3 days until the end of the experiment. The plant water requirement for L_{60}, L_{80}, L_{100}, and L_{120} was obtained by applying coefficients of 0.6, 0.8, 1, and 1.2 to the amount of plant water requirement (L_{100}) and added to the pots. Weed control operations were performed manually until the harvest stage.

Results and Discussion

The values of MWD_{wet}, MWD_{dry}, GMD, AS, WSSI, and NSI increased with the consumption of humic acid and irrigation levels up to 100% of plant water requirement (L_{100}) and decreased at the L_{120} treatment (Figure 1a, b, c, d, e, f). The highest values of MWD_{wet}, MWD_{dry}, and GMD were observed in the $L_{100}HA_4$ treatment, which was 49.92%, 74%, and 67.01% higher, respectively, compared to the $L_{60}HA_0$ treatment (Figure 1a, b, c). These values for AS, WSSI, and NSI were 2.04, 3.30, and 2.46, respectively (Figure 1e, d, f). The maximum value of PAD was observed in the $L_{60}HA_0$ treatment with a 32.9% increase compared to the $L_{100}HA_4$ treatment (Figure 1g). Blanco-Canqui *et al*., (2010) reported the MWD of soil under deficit irrigation, significantly increased with increasing the soil organic carbon content. Huisz *et al.*, (2009) investigated the effect of irrigation levels and nitrogen fertilizer on soil aggregate stability, and reported that high irrigation level increased stability indices such as NSI and MWD. Conversely, high moisture content has been reported as a destabilizing factor for soil aggregates, as excessive irrigation breaks down aggregates into smaller particles (Emerson, 1997).

In general, organic matter is one of the key factors in increasing soil particle stability and developing soil structure (Fonteyne *et al.*, 2021; Zhou *et al.*, 2022). Humic acid, by maintaining soil moisture and improving soil structure, can increase soil porosity (Chen *et al.*, 2021). Zhu *et al.*, (2018) reported that adding crop residues (SOM) humic acid contributed to the soil particle stability. Tian et al. (2019) examined the effect of humic acid on soil particle stability and reported that MWD and GMD values in humic acid treatments were 33% and 23% higher, respectively, than the control. Additionally, Manas *et al.*, (2020) observed that calcium humate and humic acid significantly improved MWD compared to digestive treatments. Shao *et al.*, (2019) reported that increasing the consumption of soil organic matter increased the content of organic materials, including humic acid, resulting in an increase in MWD_{wet} and a decrease in PAD. Zhao *et al.* (2022) studied some organic materials, including vermicompost, bagasse, biochar, and humic acid, on the stability of soil particles. They found that the treatment containing humic acid had the lowest percentage of particle destruction and the best overall soil stability.

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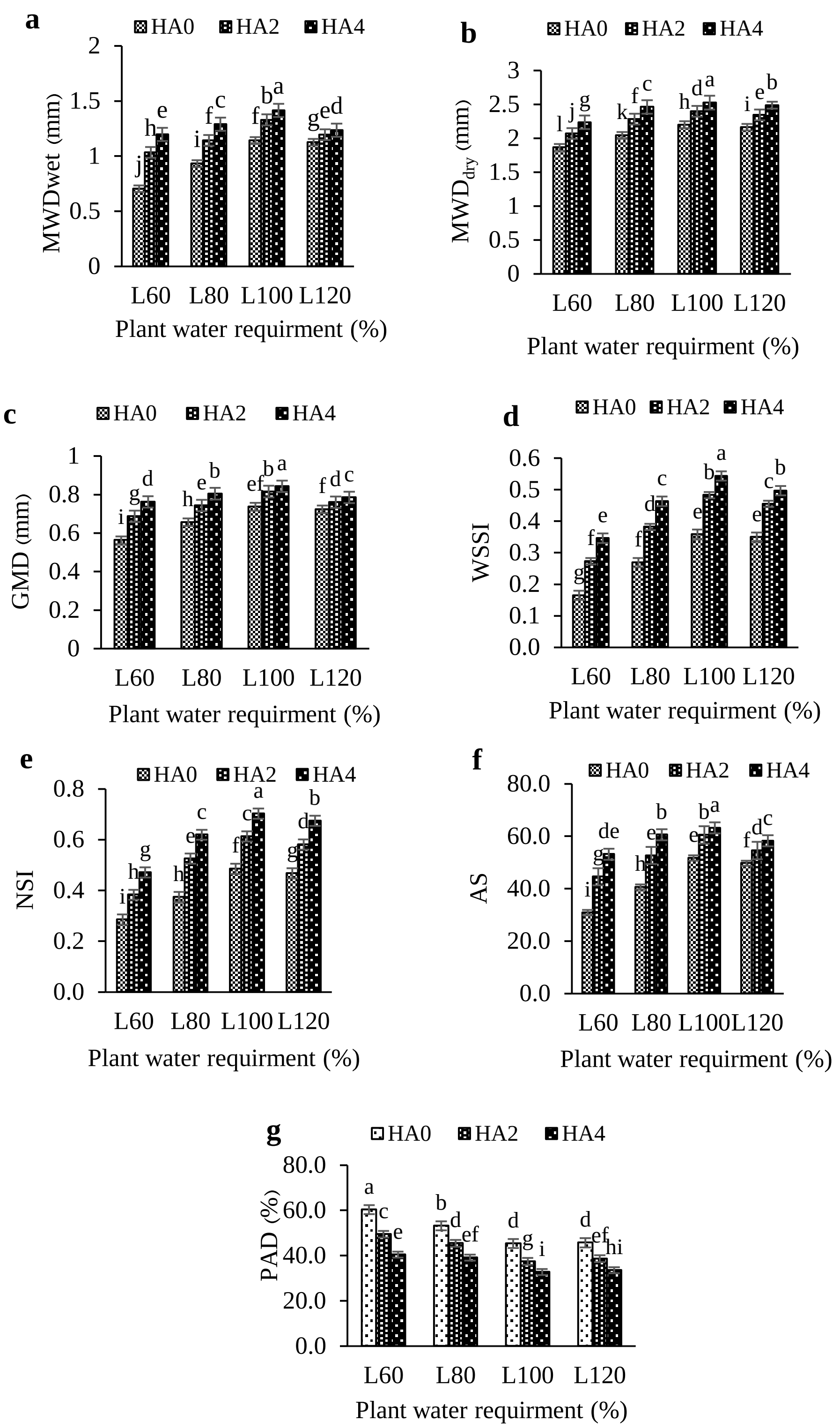

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Figure 1: Interaction plant water requirement and humic acid on soil particle mean weight diameter in wet state (a), soil particle mean weight diameter in dry state (b), geometric mean diameter of soil particles (c), whole-soil stability index (d), normalized stability index (e), soil particle stability (f), and percentage of soil particle destruction (g).

The effect of soil water content and humic acid on microbial biomass carbon (MBC), urease, and alkaline and acidic phosphatase activities is shown in Figure 2. According to the results, with an increase in the amount of humic acid and soil water content to 100% of the plant water requirement, the values of microbial biomass carbon, and alkaline and acidic phosphatase activities increased, and then decreased with increasing the soil water content (L_{120} treatment). Moreover, with an increase in the amount of humic acid and water up to the L_{80} treatment, urease activity increased, and then decreased with an increase in soil water content up to the L_{100} and L_{120} treatments. However, enzyme activities in the L_{80} treatments did not show a significant difference compared to the L_{100} treatment. Highest microbial biomass carbon content (168.40 mg C/Kg soil), and alkaline and acidic phosphatase activities (845.54, and 671.30 µg PNP g^{-1}dry soil h^{-1}) were observed in the L_{100} treatment and highest urease activity (678.98 µg NH_{3+} N g^{-1} dry soil $2h^{-1}$) was observed in the L_{80} treatment, which were 4.6, 2.8, 6, and 5.9 times higher than the $L_{60}HA_0$ treatment respectively. The acidic phosphatase enzyme activity in the $L_{60}HA_4$ treatment (350.50 µg PNP g^{-1} dry soil h^{-1}) was 3.15 times higher than the $L_{60}HA_0$ treatment. Thus, it can be concluded that a decrease in soil moisture and the presence of stress drought affect enzymatic activity. Adding humic acid had the best effect on enzymatic activity, leading to increased stability and establishment of the microbial community.

The application of organic fertilizers can be considered a factor in increasing enzymatic activity, promoting microbial growth, and enhancing root exudation. Humic acid, by improving water retention in the soil, mitigates moisture stress conditions in the soil and enhances enzymatic activity under stress conditions. Researchers have reported that adding organic matter and increasing root exudates result in enhanced access of microorganisms to nutrients, leading to increased phosphatase activity. Additionally, organic matter, such as humic acid, increases phosphatase activity in the soil through mineralization processes, providing more phosphorus in the soil solution.

Moisture can directly and indirectly affect soil microbial and enzymatic activities. The results showed that microbial activity decreased when soil moisture approached saturation due to limited oxygen availability. It has been shown that a 21% reduction in soil moisture can lead to a 42-60% decrease in urease enzyme activity. Humic acid consumption increases enzymatic activity and improves microbial growth. It enhances enzymatic activity by positively influencing biological activity. Humic acid improves soil porosity, enhances the soil's ability to retain

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moisture, and positively influences phosphatase enzyme activity by maintaining a positive relationship between soil porosity and enzyme effectiveness.

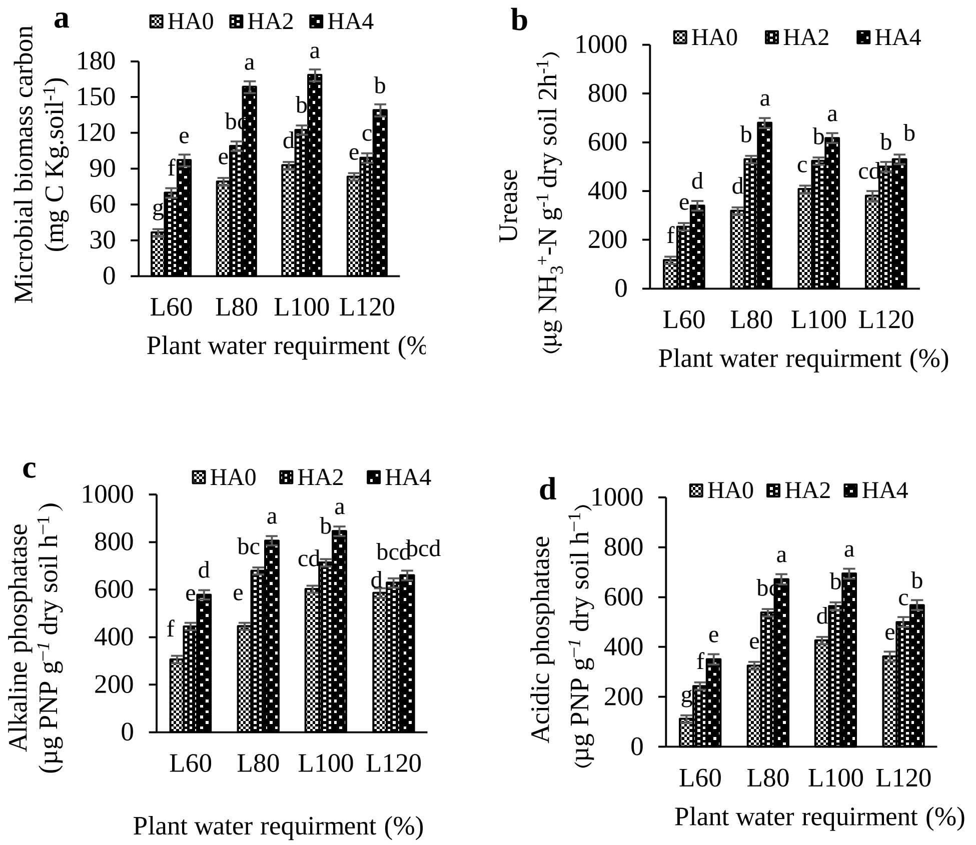

Figure 2: Interaction plant water requirement and humic acid on microbial biomass carbon (a), urease (b), alkaline (c), and acidic phosphatase (d).

Water Use Efficiency (WUE)

According to the results, with an increase in the application of humic acid, the yield of bell pepper increased (Figure 3a), and water use (WU) decreased (Figure 3b), increasing WUE (Figure 3c). The highest water consumption (7136.80 m^3/ha) was observed in the $L_{120}HA_0$ treatment. Additionally, the lowest yield (21.14 g/plant) and water use (3584.61 m^3/ha) were observed in $L_{60}HA_0$ and $L_{60}HA_4$, respectively.

Furthermore, the results showed that the maximum WUE (7.45 Kg/m^3) was obtained in $L_{80}HA_4$, while the minimum WUE (0.80 Kg/m^3) was observed in $L_{60}HA_0$ (Figure 3c). Bell peppers can increase WUE through changes in plant physiology such as stomatal closure and reduced transpiration, leading to increased yield under conditions of reduced moisture (Zakki 2020).

Zakki (2020) reported that bell peppers under drought stress improved WUE by maximizing yield. Moreover, the use of organic materials in the soil is an essential strategy to enhance performance quality and WUE. The presence of humic substances plays a role in improving stomatal conductance and helps preserve water in plants under dry stress conditions by closing stomata (Aguiar *et al.*, 2016). Humic acid as a soil organic matter can be effective in altering soil hydraulic properties, improving soil aggregation, increasing water retention capacity, and enhancing WUE (Qin *et al.*, 2020). In this regard, Alenazi *et al.*, (2016) demonstrated that the combination of humic acid and fulvic acid (FA), in addition to improving potato performance, increased WUE. Additionally, Qin and colleagues (2018) reported that humic acid reduced leaf stomatal conductance and transpiration under low irrigation intensities (20% and 40%) and assisted in pepper growth by reducing soil moisture loss.

Ali *et al.*, (2019) studied the effect of humic acid application and different irrigation levels (60%, 80%, and 100% of standard evapotranspiration (ETc)) on Water Use Efficiency (WUE) in potato cultivation. They reported that the highest water use efficiency was observed in the presence of humic substances (HA) and 60% standard evapotranspiration treatments. El-Hashash *et al.*, (2022) used humic acid (0, 30, and 60 Kg/ha) under full plant water requirement and drought stress conditions in wheat cultivation, and they identified the highest energy use efficiency and plant performance in HA_{60} treatment. Qin *et al.*, (2020) studied the effect of water deficit and the use of humic acid in watermelon cultivation. The results showed that a 25% to 50% reduction in irrigation led to a partial decrease (15-8%) in crop yield, resulting in higher water use efficiency.

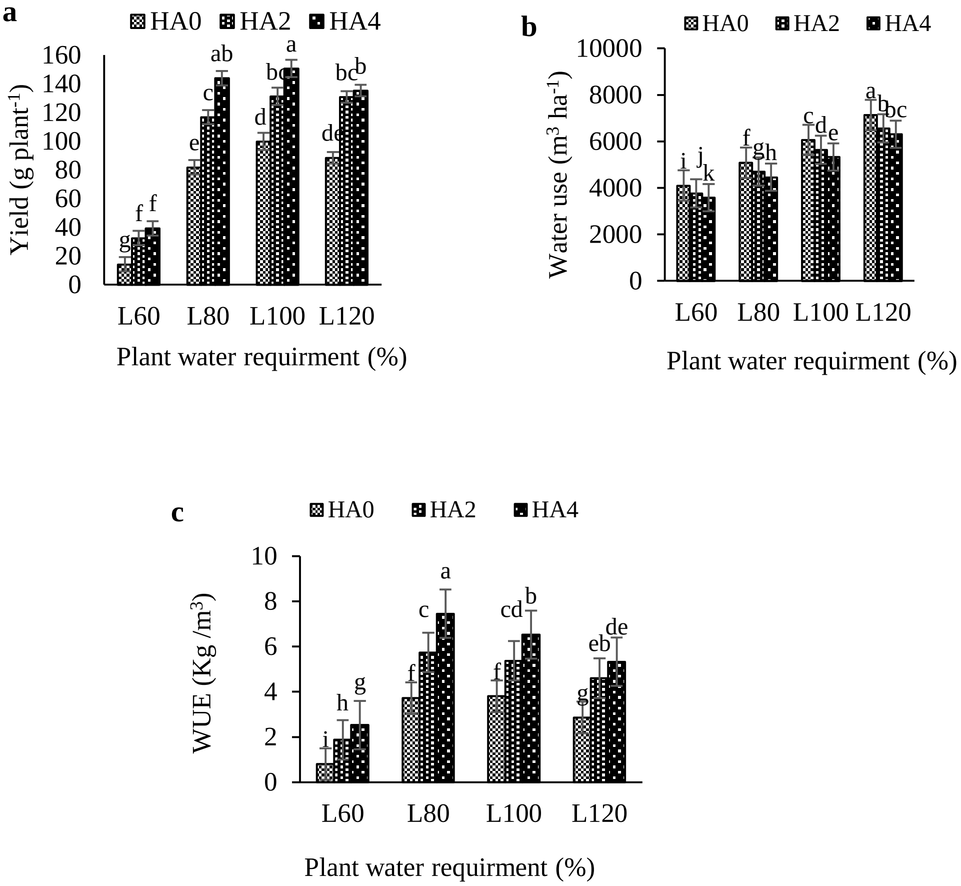

Figure 3: Interaction plant water requirement and humic acid on the performance (a), water consumption (b), and water use efficiency (c) in bell pepper plants (*Capsicum annum* L).

Conclusion

The Results showed that the application of humic acid and a 20% reduction in the water requirement of bell pepper cultivation (L_{80} treatment compared to treatment L_{100} treatment) led to an increase in water use efficiency in the soil. Additionally, the highest activity of soil microorganisms, microbial carbon biomass, acidic and alkaline phosphatase enzymes were observed when 100% of the bell pepper water requirement was applied with the application of 4 grams of humic acid per kilogram of soil ($L_{100}HA_4$ treatment). However, the maximum soil stability indices (MWD_{wet}, MWD_{dry}, GMD, AS, WSSI, NSI), were observed in conditions where the plant's water requirement was fully met and the maximum percentage of soil particle destruction (PAD) was obtained in $L_{60}HA_0$ treatment. The results of this study demonstrate that the use of humic acid can be highly effective in reducing the water

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requirements of bell pepper plants in greenhouse conditions under experimental conditions. In general, the results showed that the use of humic acid (especially the use of 4 g of humic acid per Kg of soil (HA_4 treatment) and less water use (L_{80} treatment) improves physical and biological characteristics such as increasing soil enzyme activity, soil respiration, soil stability and reduced the percentage of destruction of soil aggregates. Therefore, considering the severe crisis of water scarcity in most parts of Iran, relying on the results of the present research, the simultaneous use of humic acid and reducing the water requirements of bell pepper plants can be a valuable strategy to improve the physical and biological characteristics of the soil and save the water consumption in greenhouse conditions.

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