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Effects of Vermicompost on Growth Parameters, Water Use Efficiency and Quality of Zinnia Bedding Plants (*Zinnia elegance* 'Dreamland Red') under Different Irrigation Regimes

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Abstract

The drastic decline of precipitation over the past decade along with overuse of chemical inputs and consequent soil fertility reduction are the most important problems in the approach to agricultural expansion, particularly in urban landscaping. Therefore, an experiment was designed to examine the effects of different irrigation levels (40, 70 and 100 % of field capacity, or FC) and different vermicompost (VC) levels (0, 2.5 and 5 %) on the morphophysiological parameters of *Zinnia elegance* 'Dreamland Red' in a factorial experiment based on completely randomized design with three replications. Results indicated a significant reduction in root/shoot ratio, flower diameter, flower longevity, water use efficiency (WUE), photosynthetic pigments and nutrient uptake, along with reduction in the FC, while VC application improved these features. Based on the results, the highest electrolyte leakage and free radicals were observed in the 40 % of FC level without VC treatment, while the highest antiradical property and phenolic compounds were obtained at the same FC level along with 2.5% VC. Moreover, the highest WUE was observed at 2.5% VC with 70% FC, compared to 40% FC with no VC application. The flower diameter and longevity, as the most important indicators of the zinnias' quality, were significantly affected by the interaction effects of irrigation and organic fertilizers.

Keywords: antiradical properties, field capacity, phenolic and flavonoid compounds.

Introduction

Drought and salinity are the most important influencing parameters in the growth of plants in dry and semi-dry climates (Ehsanpoure and Razavizadeh, 2005). According to the results of various studies, the stress imposed by lack of water leads to the decrease in growth of different parts of the plant, including roots and shoots (Hung *et al*., 2005), photosynthesis and chlorophyll (Akhkha *et al.,* 2011) and mineral uptake (macro- and micronutrients) (Shamshiri *et al*., 2011). On the other hand, overuse of chemical fertilizers in the fields, besides increasing costs, has caused irreversible effects on the environment (soil and water contaminations) and human health (Mirzaei *et al*., 2009). Thus, with respect to the decrease in the organic materials in dry climates (Melero *et al*., 2008), the replacement of organic fertilizers with chemical fertilizers in recent years in the production of horticultural crops, such as bedding plants, with the use of organic fertilizers (such as vermicompost) is an inevitable necessity. Vermicompost (VC) is produced by decomposition of organic

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matter during a non-thermal process, through interaction between earthworms and microorganisms (Chen *et al*., 2004). Vermicompost, due to its possession of humic substances (Chen *et al*., 2004), absorbable minerals, vitamins, hormones and different enzymes (Prabha *et al*., 2007), leads to improvement of physical (porosity and water and nutrient-holding capacity) (Jashankar and Wahab, 2004) and of the chemical properties of cultivation substrate (cation exchangeable capacity and minerals) (Atiyeh *et al*., 2001a). The advantages of VC application in comparison to other organic fertilizers include the nutrients' availability (Mamo *et al*., 1998), performance of the enzymes (protease, amylase, cellulase and pectinase), microorganisms and different hormones, and the consequent decomposition of organic materials and increase in the availability of nutrients the plants need (Jat and Ahlawat, 2004 and 2006). The studies conducted on *Dieffenbachia* showed increases in the absorption of nitrogen, phosphorus, potassium, calcium and magnesium of the leaf under the effect of VC (Khomami, 2010).

Given the small number of studies undertaken on the effect of vermicompost on growth of some horticultural crops, very little information is available on the effect of this fertilizer on the growth and cultivation of flowers, especially bedding plants, with regard to the fact that there is a strong relationship between irrigation and fertilization and the quality of these flowers. Thus, the main aim of this study was to investigate the effect of VC on the improvement of nutrient absorption without using chemical fertilizers on some morphological parameters of *Zinnia elegance*, particularly the quality of the produced flowers and the efficiency of water consumption under conditions of water scarcity.

Materials and Methods

This pot and open field experiment was conducted during the spring and summer of 2012 with an average daily temperature of 29ºC and 45% relative humidity, on the *Zinnia elegance* 'Dreamland Red' in a factorial experiment (3×3) based on a completely randomized design with three replications, in order to evaluate its response to vermicompost (VC) (0, 2.5 and 5%, W/W) under different soil moisture levels [40, 70 and 100% of field capacity (FC)]. The soil (sandy loam texture with 0.5% organic matter) after inoculation by different VC levels was transferred to the 3-L volume pots, and transplanting was done at the four leaves phase.

Irrigation regimes were applied based on the field capacity (FC) method (soil moisture content); briefly, 48 h (depending on soil texture) after full irrigation to the saturation point of each treatment, and after drainage of gravity water, each pot was weighed and considered as FC 100 %. Also, other FC levels were calculated accordingly (Henry, 1990). All FC levels (every day during the experiment) were separately and thoroughly checked, weighed and irrigated to the end of the flowering period.

Growth parameters such as shoot dry weight, root/shoot ratio, flower diameter, flower longevity (from anthesis to flower wilting) and water use efficiency (WUE) (Karkanis *et al*., 2011) were measured during the experiment.

The total chlorophyll content of young mature leaves at the flowering stage was determined after extraction in 80% acetone (Gross, 1991). Absorption was measured using a spectrophotometer (Perkin Elmer, UV/VIS, Lambda 25), at 645 and 663 nm and thus total chlorophyll was determined.

Cell membrane stability was measured using an electrical conductivity meter based on the method of Lutts *et al*. (1996). Leaf samples were placed in individual stoppered vials containing 25 ml of deionized water. These samples were incubated at room temperature (25°C) on a shaker (150 rpm 30 min^{-1}). The electrical conductivity of the solution (EC1) was read after shaking. Samples were then placed in a thermostatic water bath at 95°C for 15 min^{-1}, and the second reading (EC_2) was determined. Membrane permeability was calculated as EC1/EC2 and expressed as a percentage.

Total phenol was measured according to Folin–Ciocalteu reagent (Pourmorad *et al*., 2006). Sample extracts (1 g DW 10 ml^{-1} ethanol) of each treatment or gallic acid (standard phenolic compound) was mixed with Folin–Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and aqueous Na_2CO_3 (4 ml, 1 M). The mixture was allowed to stand for 15 min and the total phenols were determined by colorimetric at 765 nm. Total phenol values are expressed in terms of gallic acid equivalent (mg g^{-1} of dry mass), which is a common reference compound.

The stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) was used for determination of free radical-scavenging activity (Koleva *et al*., 2002). After addition of the treatment's extract to methanolic solution of DPPH (100 M, 15 min at room temperature), the absorbance was recorded at 517 nm. BHT and quercetin were used as standard controls. IC 50 values denote the concentration of the sample that is required to scavenge 50 % of DPPH free radicals.

Phosphate buffer (pH=7) and potassium iodide (KI/1M) were used for determination of hydrogen peroxide (Velikova *et al*., 2000). Absorbance of each sample was recorded at 390 nm, and H_2O_2 was calculated by extinction coefficient (0.28 $\mu M^{-1} cm^{-1}$) and expressed in terms of $\mu M\ g^{-1}$ fresh weight. Also, aluminium chloride colorimetric method was used for determination of the leaf and petal flavonoids (Chang *et al*., 2002), phosphorus was determined based on the Vanadate-molybdate reagent (Emami, 1996), and calcium and magnesium uptake were measured based on the titration by EDTA (0.01 M) (Ghazan-Shahi, 1997). The SAS software (version 9.1) was used to analyse the data and the mean comparisons were done with Duncan's multiple range test with probability levels of 1 and 5 %.

Results and Discussion

Shoot dry weight

The results showed a significant effect of VC, FC and their interaction on shoot dry weight (P<0.05). With the raised stress level, the dry weight of shoot showed a decreasing trend; however, with inoculation of substrate with VC, the shoot dry weight increased (Table 1). However, with high levels of drought stress, the addition of VC did not prevent the reduction of biomass. This may be due to competition between soil and VC particles for water in severe stress conditions (FC 40%), which led to lower water availability for the zinnia, and consequently lower biomass production. Achieving the minimum biomass produced in the lowest irrigation regime (40% FC) may be due to the decrease in the water consumed by the plant, thus leading to the downward trend in the absorption of nutrient elements such as phosphorus, and also to the increase in the amount of free radicals and decrease in the dry matter. This is in agreement with the results of the studies conducted by Bachman and Davis (2000) on magnolia, which examined the improvement of the dry weight of plants treated by VC.

Table 1. Effects of irrigation regime (FC) and vermicompost (VC) on shoot and root to shoot ratio of Zinnia 'Dreamland Red'.

FC (%)	VC (%)	Shoot dry weight (g DW/plant)	Root to shoot ratio
100	0	$1.77^{b\dagger}$	0.12^{bc}
	2.5	2.15^{ab}	0.1^{bc}
	5	2.02^{ab}	0.09^{c}
70	0	2.27^{ab}	0.13^{bc}
	2.5	2.44^{a}	0.2^{a}
	5	2.37^{a}	0.14^{b}
40	0	1^{c}	0.1^{c}
	2.5	1.1^{c}	0.12^{bc}
	5	0.94^{c}	0.09^{c}

[†] Means are marked with the same letters; do not have a significant difference according to Duncan's multiple range test in probability level of 5%.

Root/shoot ratio

FC, VC and their interaction showed significant effects on the root/shoot ratio in zinnia ($P<0.05$). The highest root/shoot ratio was observed with the 70% irrigation regime (FC) and 2.5% treatment of VC (Table 1). In FC 100%, with an increase of VC up to 5%, the growth of shoots compared to roots increased by comparison to the control. However, in treatments of 40% and 70% field capacities, adding 2.5% vermicompost led to an increase, and treatment of 5% VC caused a decrease in root/shoot ratio; this in agreement with the studies of Abdalla and Khoshiban (2007), indicating a decrease in the dry weight of shoots due to an increase in drought stress. In the water deficit conditions, the aerial plant parts are influenced more than the roots. In other words, the growth of aerial parts stops sooner than the roots, which in turn causes an increase in the root/shoot ratio (Chiatante *et al*., 2006). Soils containing VC cause an increase in the growth of the plant, due to the existence of minerals (Mamo *et al*., 1998). In addition to the improvement of soil field capacity, one of the reasons for the improvement of root/shoot ratio under the influence of VC in this experiment is probably the provision of more and better mineral nutrients.

Flower diameter and flower longevity

In comparison to VC treatment, FC treatments showed a greater impact on zinnia flower diameter (Fig. 1A). Accordingly, in the 70% and 100% levels of FC and 2.5% VC, despite the increase in flower diameter in comparison to control (without VC), no significant difference was observed between VC treatments; nevertheless, with an increase of stress level up to 40% FC, the flower diameter significantly decreased, and adding VC at 5% level prevented flowering in the described treatment. This is probably due to the very deficit irrigation condition and burns caused by the excessive amount of fertilizers (VC).

The trend of response in zinnia's flower longevity to the irrigation regime and VC was different. Accordingly, flower durability of the plant at 100% FC treatment with 2.5% VC added was significantly increased compared with the control (without VC) (Fig. 1B). This trend was observed at 70% FC with no significant difference between treatments, while in the high drought stress treatment (40% FC), adding VC resulted in a significant decrease in the zinnia's flower longevity. A decrease in flower longevity under high drought stress, coupled with added VC, can be due to severe water deficit and also the competition between VC fertilizer, soil colloids and the roots to absorb water and, thus, the decrease in water availability and insufficient water absorption by the root. With respect to the role of phosphorus as one of the most important and effective elements in flowering (Taiz and Zeiger, 2003), and also the effect of calcium in cell stability (Barker and Pilbeam, 2007) and delay in flower senescence (Ferguson, 1988), the results of this experiment indicated an improvement in absorption of phosphorus and calcium elements with high FC levels and VC compared to the control (Table 3). Thus, improvement of the uptake of the abovementioned minerals is considered an effective factor in the improvement of diameter and zinnia flower longevity. Atiyeh *et al*. (2001b and 2002) stated that the activities of the microorganisms present in the VC lead to improvement in absorption of minerals, and consequently to an increase in the flower diameter and length of flowering period in marigold flowers; the results of this experiment suggest a similar pattern.

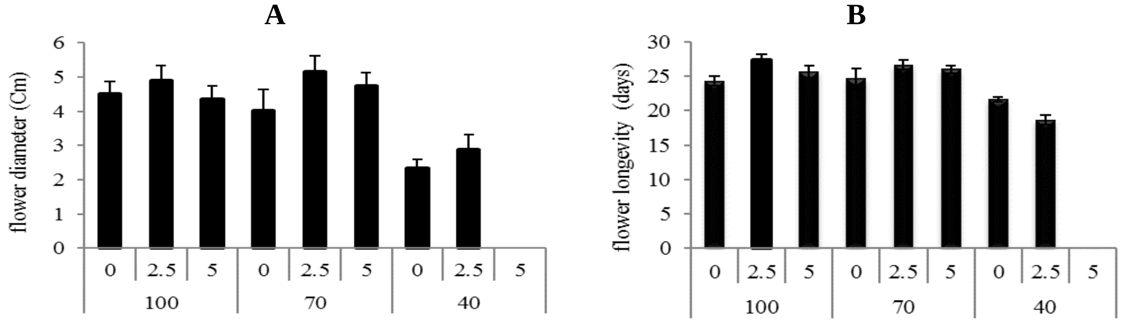

Fig. 1. Effects of vermicompost on flower diameter (A) and flower longevity of 'Dreamland Red' (B) zinnia under different FC levels.

Water use efficiency (WUE)

The results obtained indicated a significant interaction effect of irrigation regime and VC on WUE ($P<0.05$) (Fig. 2). Thus, the highest WUE was observed in the 2.5% VC and 70% irrigation regime. Maximum WUE in 2.5% VC and 70% FC treatments can be attributed to the maximum biomass produced in this treatment (Table 1) considering the balanced water consumption in comparison to other treatments. In fact, considering the dry material produced in exchange for the water consumed, or the WUE index, it can be concluded that in this experiment, adding VC to the substrate leads to an increase in water maintenance and thus to an increase in expansion of the plant root, leading to an increase in water and nutrients uptake and finally to improvement in dry material production, especially in balanced irrigation conditions. Improvement in WUE depends on the increase in performance or decrease in evaporation and transpiration (Sohani, 2000). Gindaba *et al.* (2005) reported a decrease in WUE due to an increase in drought stress in greenhouse conditions for plants such as *Cordia millettia* and *Croton macrostachyus.*

Total chlorophyll content

The total chlorophyll content demonstrated a downward trend in response to a decrease in FC; however, VC treatment led to a decrease in this downward trend (Table 2).

The delay in the destruction of photosynthetic pigments under the effects of VC can be attributed to improved water efficiency. Considering the results of this study, increase in drought stress decreases WUE and leaf relative water content, and may bring about destruction of chloroplast and chlorophyll loss. The investigation of magnesium (Mg^{++}) uptake in substrates treated with VC under drought stress conditions and 100% FC shows an increase in Mg^{++} uptake as a positive element in stabilizing chlorophyll (Taiz and Zeiger, 2003) compared to control (without VC); this is another explanation of vermicompost's moderating role in the above mentioned conditions. The application of vermicompost fertilizer led to an increase in the amount of chlorophyll in pistachio seedlings, which is in line with the results of this research (Golchin *et al.*, 2006).

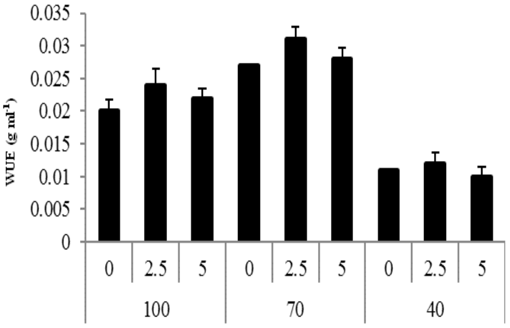

Fig. 2. Effect of vermicompost on water use efficiency (WUE) of 'Dreamland Red' zinnia under different FC levels.

Table 2. Effects of irrigation regime (FC) and vermicompost (VC) on some physiological traits of *Zinnia* 'Dreamland Red'.

FC (%)	VC (%)	Total chlorophyll ($g\ L^{-1}$)	Ion leakage (%)	Free radical ($\mu M\ g^{-1}$ FW)	Antiradical properties (%)
100	0	0.00126^{a}[†]	39.2^{d}	11.9^{f}	80.4^{d}
	2.5	0.00129^{a}	27.9^{f}	10.6^{f}	86.2^{c}
	5	0.00127^{a}	28.1^{f}	11^{f}	86^{c}
70	0	0.00073^{bcd}	43.4^{c}	16.5^{d}	89^{bc}
	2.5	0.0011^{ab}	32.1^{e}	14.2^{e}	92.1^{ab}
	5	0.00097^{abc}	32.4^{e}	14.3^{e}	91.7^{ab}
40	0	0.00039^{de}	57.6^{a}	24.9^{a}	94.7^{a}
	2.5	0.00059^{cde}	46.7^{b}	18.4^{c}	94.8^{a}
	5	0.000177^{e}	46.9^{b}	21.7^{b}	93^{ab}

[†] Means are marked with the same letters; do not have a significant difference according to Duncan's multiple range test in probability level of 5%.

Cell membrane stability, hydrogen peroxide and antiradical properties

According to the results, cell membrane stability was affected significantly by interaction effects of FC and VC ($P<0.01$). Relative water content is a good indicator of leaf water status, so that with increasing drought stress this index decreases and leads to some changes in cellular membrane, and consequently to an increase in electrolyte leakage in the cells (Fu *et al.*, 2004). Goldani and Kamali (2011) in the investigation of the effect of drought stress on ornamental *Amaranthus tricolor* and *Gomphrena globosa* showed that the drought stress leads to decrease in cellular membrane stability.

Also, FC and VC interaction significantly affects the free radical (hydrogen peroxide) and antiradical properties (Table 2). On these bases, the highest amount of free radicals was observed in severe drought stress (40% FC) and without VC treatment, while the lowest amount was observed at 100% FC and 2.5% VC level. The increase in free radicals in reaction to drought stress (Mafakheri *et al.*, 2010), along with the increase in defence power and production of antioxidant and antiradical compounds in reaction to drought stress (Jaleel *et al.*, 2007), with the decrease of undesired effects caused by free radicals, are all in line with the findings of this study regarding decrease in water uptake and consequent increase in free radicals in FC of 40%. It is clear that the application of VC in the substrate leads to improvement in the strong root system and aqueous conditions, and consequent decreases in the undesired effects caused by severe stress and oxidative in treated plants in comparison to control. Garcia *et al.* (2012), in their investigation of VC in rice seedlings, reported that the VC treatments led to a decrease in hydrogen peroxide compared to the control (without VC), which is in agreement with the results of this study.

Total phenol and flavonoids in leaves and petals

Table 3 shows that increase in drought stress (40% FC) and application of VC (2.5%) leads to an upward trend in leaves' total phenol and petal flavonoid content compared to control (100% FC and without VC). Increased total phenol and flavonoid contents can be evaluated in terms of the fact that the decrease in irrigation regime, increasing drought stress as oxidative stress (Sofo *et al.*, 2004), leads to destruction of cellular membranes (Fu *et al.*, 2004), and that the increase in free radicals (Mafakheri *et al.*, 2010), on the one hand, and the stimulation of defence mechanisms, and enzymatic (catalase and peroxidase; Mittler, 2002) and non-enzymatic (phenolic compounds; Delitala *et al*., 1986; alkaloids; Szabo *et al*., 2003) antioxidant activities, on the other hand, occur to modulate stress and improve the defensive mechanism of the plant. This is in line with the findings of this study regarding the increase in free radicals resulting from drought stress and the increase in phenolic compounds, as an antioxidant system, which occur to mitigate the undesirable effects of drought stress. Saneoka *et al*. (2004), in their investigation of olive plants under drought stress conditions, reported an increase in phenylalanine ammonia lyase (PAL) enzyme activity and total phenol content. Of course, the application of VC in the substrate, depending on concentration, leads to an increase in total phenol and flavonoid content, which is probably due to higher availability of nutrients such as phosphorus and calcium, and the improvement of the conditions for appropriate reactions to drought stress. Cakmak (2000) reported that with micronutrient deficiency, the activity of antioxidant enzymes decreases; therefore, the plant's sensitivity to environmental stress increases.

Table 3. Effects of irrigation regime (FC) and vermicompost (VC) on phenolic compounds and mineral uptake of *Zinnia* 'Dreamland Red'.

FC (%)	VC (%)	Total phenol (mg g^{-1} DW)	Leaf flavonoid (mg g^{-1} DW)	Petal flavonoid (mg g^{-1} DW)	P (%)	Ca (%)	Mg (%)
100	0	$40.85^{fg\dagger}$	2.95^{fg}	0.78^{e}	0.75^{ab}	0.4^{a}	1.2^{ab}
	2.5	44.51^{f}	3.73^{f}	1.05^{e}	0.84^{a}	0.56^{a}	1.36^{a}
	5	38.85^{g}	2.49^{g}	0.92^{e}	0.8^{ab}	0.53^{a}	1.4^{a}
70	0	87.85^{e}	9.61^{e}	4.35^{d}	0.44^{c}	0.2^{b}	0.83^{bc}
	2.5	94.85^{d}	13.68^{c}	5.14^{c}	0.71^{ab}	0.5^{a}	1.23^{ab}
	5	92.01^{de}	12.09^{d}	4.72^{cd}	0.66^{b}	0.43^{a}	1.1^{ab}
40	0	110.01^{c}	16.05^{b}	7.82^{b}	0.25^{d}	0.13^{b}	0.53^{cd}
	2.5	127.35^{a}	18.85^{a}	9.39^{a}	0.42^{cd}	0.16^{b}	0.57^{cd}
	5	122.01^{b}	18.24^{a}	0^{f}	0.4^{cd}	0.073^{b}	0.38^{d}

[†] Means are marked with the same letters; do not have a significant difference according to Duncan's multiple range test in probability level of 5%.

Mineral uptake

Uptake of minerals was significantly affected by FC, VC and their interaction effects ($P<0.05$). With an increase of drought stress, a downward trend in absorption of the abovementioned elements was observed; however, application of VC especially at the 2.5% level led to a decrease in the downward trend of mineral uptake (Table 3). Absorption of calcium, as an immobile element, occurs through a passive process and is very strongly influenced by the aquatic condition of the substrate. Dryness also has a significant effect on phosphorus uptake (Mahmoudi and Hakimian, 2007). Considering the greater increase in accessibility of nutrients as a result of VC application treatment (Mamo *et al*., 1998), besides the higher availability of these elements by VC fertilizer, the increase in nutrient uptake under the desirable conditions of FC and VC can also be attributed to the sufficient availability of water, and the appropriate volume and development of the root, and thus the better absorption of the nutrients from the substrate. The results of this study are in line with the results of Khomami (2010), which reported that increase in VC leads to increase in phosphorus, potassium, calcium and magnesium in *Dieffenbachia*.

Conclusions

Based on the results of the present study and the decrease in the qualitative characteristics of the *Zinnia elegance* in low irrigation regimes, especially at 40% field capacity, and the improvement of growth conditions and nutrient uptake under VC treatment, the application of vermicompost at 2.5% is recommended to improve the qualitative properties and decrease losses due to deficient irrigation in *Zinnia elegance* ‘Dreamland Red’

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