

Growth and Physiology of *Chrysanthemum morifolium* Supplemented with Various Fertilizers

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To evaluate the effectiveness of various organic or inorganic fertilizers on the growth and physiology of *Chrysanthemum morifolium* (an important ornamental plant), the present research was conducted in a completely randomized design. Seedlings were treated with vermicompost (0 and 40% w/w of soil), nano-chelated zinc (nano-Zn) (0 and 0.1% (w/v)) or ZnSO₄ (0 and 0.2% (w/v)). Nano-Zn or ZnSO₄ were sprayed three times at two-week intervals. In comparison to the control, the applied fertilizers significantly promoted growth rates and biomass accumulations as indicated by the significantly higher leaf area as well as leaf fresh and dry mass in the treated plants by approximately 41%, 39%, and 28%, respectively. The simultaneous applications of nano-Zn and vermicompost the most effective supplementation to improve plant growth rate. The combined applications of mineral and biological fertilizers led to significant increases in the contents of photosynthetic pigments by about 53%. Except for individual application of ZnSO₄, the other applied treatments, especially the combined ones, resulted in significant increases in the contents of leaf proline (by, on average, 51%) as compared to control. Similarly, the simultaneous applications of supplements increased the root proline by 82%. The highest amounts of leaf soluble phenols were found in nano Zn-V group (3.3 folds higher than control). However, in root tissues, the vermicompost treatment was the only source of significant increases (by approximately 49%). In conclusions, the simultaneous soil supplementations with the biological fertilizers and foliar applications of nano-fertilizers may be regarded as a suitable eco-friendly way to improve plant metabolism and growth rates.

Abstract

Keywords: Compatible osmolites, Nanofertilizers, Nutrition, Ornamental, Vermicompost.

INTRODUCTION

Improvements in crop growth rate, qualities and yield are obviously a critical goal of sustainable agriculture (Doan *et al.*, 2015). Various industrial and agricultural practices, e.g. the application of chemical fertilizers, have improved crop yields at the expense of soil quality (Doan *et al.*, 2015) so that soil organic matter and biodiversity have been lost (Papathanasiou *et al.*, 2012). Recently, interests have been aroused in organic farming as an eco-friendly method in which the chemical fertilizers and pesticides are mainly avoided (Nurhidayati *et al.*, 2016).

Vermicompost is a product of the vermicomposting process catalyzed by earthworms (Ansari and Sukhraj, 2010). It is known as an eco-friendly way of biological degradation of organic wastes and production of biological fertilizers (Edwards and Burrows, 1988). It possesses growth-promoting compounds, especially humic substances, thereby improving plant growth, enhancing water holding capacity and microbial growth in soil (Bhat and Limaye, 2012). Vermicompost provides suitable conditions for plant nutrition, mainly via the slow and steady release of mineral nutrients (Ansari and Sukhraj, 2010). It has been documented that different kinds of vermicompost may improve the availability of minerals and modify various physicochemical and microbiological characteristics of the soil (Atiyeh *et al.*, 2000). There are many studies introducing vermicompost as an excellent soil conditioner that may improve qualities of different crops such as tomatoes (Gutiérrez-Miceli *et al.*, 2007) and Chinese cabbage (Wang *et al.*, 2010). However, the effect of vermicompost on the plant growth rate and yield is highly variable, mainly depending on the type of cultivation system and the properties of the applied vermicompost (Nurhidayati *et al.*, 2016).

Nowadays, novel applications of nano-particles are rapidly developing in the different sciences and industries (Alharby *et al.*, 2016). Plant responses to various nano-compounds actually vary depending on size and concentration of nano-particle, the plant species, and soil type (Dietz and Herth, 2011; Yang *et al.*, 2015; Peralta-Videa *et al.*, 2014). Zinc (Zn) that is known as a vital essential micronutrient implicates in the various aspects of plant cell metabolism (Taiz and Zeiger, 1994). The deficiency of Zn is widespread throughout the alkaline and calcareous soils (Rashid and Ryan, 2004). Due to its less mobility and higher rates of soil fixation, the foliar application is a more effective way of relieving signs of Zn deficiency than soil application. Both phytotoxicity and benefits of nano-particles have been reported in different plants. The inhibiting effects of nano zinc oxide have been recorded in maize and rice (Yang *et al.*, 2015).

Nowadays, there is more interest to introduce different alternative eco-friendly methods for keeping soil fertility, mitigating the environmental impacts of various agricultural activities, and improving plant growth and tolerance. The current research was conducted to evaluate and compare the responses of ornamental *Chrysanthemum morifolium* to different mineral, nano-chelated or vermicompost (biological) fertilizers. In addition, the effects of the supplements on the contents of some critical mechanisms such as phenols and proline were also addressed.

MATERIALS AND METHODS

The experimental design was based on a completely randomized design with three replications. The research was conducted in Garmsar University. Vermicompost was purchased from Gilda Company, Iran. The characteristics of the used vermicompost had been presented in our previously published research (Mousavi and Ardebili, 2014). Seedlings of *Chrysanthemum morifolium* were planted in pots containing control soil or mixed prepared soil of 40% (v/v) vermicompost. Physicochemical characteristics of the applied soil were examined, and the details are presented in Table 1.

The nano-chelated zinc was purchased from a reliable company, Khazra, Iran. Sixty-day-old seedlings were foliar treated with nano-chelated zinc at two rates (0 and 0.1% (w/v)) or ZnSO₄ (0 and 0.2% (w/v)). Nano-chelated zinc or ZnSO₄ were sprayed three times at two-week intervals. Two weeks after the last spray, the plants were harvested for physiological assessments. The treatment groups were called as follows: Control; V (vermicompost); Nano Zn (nano chelated zinc);

ZnSO₄; Nano Zn-V (the simultaneous treatment of vermicompost and nano chelated zinc); ZnSO₄-V (the simultaneous treatment of vermicompost and ZnSO₄).

Measurements of photosynthetic pigments and growth related characteristics

Photosynthetic pigments were extracted using 80% (v/v) acetone as a solvent. The determination of chlorophyll was performed according to the method of Arnon (1949). The fresh and dry masses of leaves, leaf area, and stem height were measured.

The measurement of proline contents

Proline extraction was performed using sulfa salicylic acid 3% (w/v). Its content was quantified according to the method previously described by Bates *et al.* (1973). Proline contents were calculated based on the proline standard curve and expressed in µg g⁻¹ F.W.

Determining total soluble phenols

Total soluble phenols in the leaf tissues were extracted using 70% (v/v) ethanol as a solvent and assayed using the Folin-Ciocalteu reagent method. Finally, the total soluble phenols were calculated using the standard curve of tannic acid.

Statistical procedure

The collected data were analyzed as a factorial experiment by analysis of variance using SPSS software. Means differences were determined by Duncan's multiple range test at P < 0.05.

Table 1. The physicochemical characteristics of the applied soil

Soil texture	EC (dS/m)	pH	Sand (%)	Silt (%)	Clay (%)	Total N (%)	K mg/kg	P mg/kg
Loam	3.1	7.9	50	25	25	0.9	365	14

RESULTS AND DISCUSSION

As it was presented in Fig. 1, the rates of leaf production, which is an important criterion for growth measurement, was significantly influenced by different fertilizers in various treatment groups among which the Nano Zn-V and ZnSO₄-V treatment groups, especially the former, had the highest growth rates. The highest amounts of leaf fresh and dry mass were recorded in Nano Zn-V, ZnSO₄-V, and Nano Zn treatments, respectively (Table 2). The simultaneous application of nano-zinc fertilizer and vermicompost was the most effective treatment to enhance leaf area (Table 2). The findings clearly indicated that the growth rates and biomass production were significantly affected by the applied supplements, as it was indicated by the Zn or and/or vermicompost induced modifications in leaf numbers, area, fresh weight and dry mass in treatment groups among which Nano Zn-V was related to the highest levels of different characteristics related to the growth. Therefore, the simultaneous supplementation of the plants with vermicompost and nano-chelated zinc was found to be the most effective treatment for improving the growth of *Chrysanthemum morifolium*. Also, the applied supplements significantly altered the contents of photosynthetic pigments in different treatment groups among which Nano Zn-V, ZnSO₄-V, and Nano Zn had significantly higher contents than the control (Table 3).

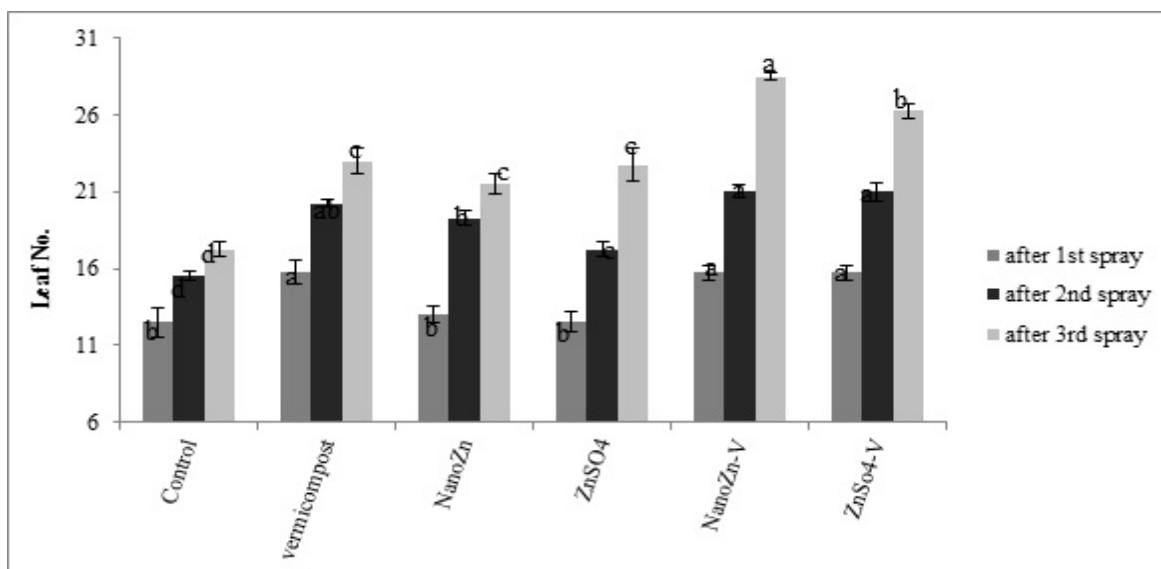


Fig. 1. The effects of the applications of nano zinc, ZnSO₄ and/or vermicompost on the numbers of the leaves recorded one week after every spray.

Table 2. The effects of different applied nutritional treatments on the leaf biomass and area

	Leaf fresh mass (g)	Leaf dry mass (g)	Leaf area (cm ²)
Control	0.38d**	0.049 ^d	10.0 ^e
V*	0.49 ^c	0.057 ^{cd}	15.2 ^b
Nano Zn	0.56 ^b	0.063 ^{bc}	13.0 ^{cd}
ZnSO ₄	0.41 ^c	0.05 ^{cd}	11.7 ^d
Nano Zn-V	0.62 ^a	0.076 ^a	17.3 ^a
ZnSO ₄ -V	0.57 ^b	0.07 ^{ab}	13.2 ^c

*: V; vermicompost

** : Data are means of three replicates. Mean values followed by different letter(s) are significantly different according to the Duncan's test.

Table 3. The effects of different applied fertilizers on the photosynthetic pigments

	Total chlorophyll (mg g ⁻¹ F.W.)	Chl. a (mg g ⁻¹ F.W.)	Chl. b (mg g ⁻¹ F.W.)
Control	3.8 ^c	1.91 ^c	1.37 ^e
V*	5.5 ^b	3.02 ^b	2.43 ^{cd}
Nano Zn	6.65 ^a	3.46 ^{ab}	2.96 ^{bc}
ZnSO ₄	3.7 ^c	1.63 ^c	2.04 ^d
Nano Zn-V	6.8 ^a	3.70 ^a	3.20 ^{ab}
ZnSO ₄ -V	6.5 ^a	3.09 ^b	3.57 ^a

*: V; vermicompost

** : Data are means of three replicates. Mean values followed by different letter(s) are significantly different according to the Duncan's test.

It is obvious that there is a positive correlation between the contents of photosynthetic amounts and photosynthesis, thereby improving the plant growth rate. Zn is an essential oligo-element that plays a critical role in the synthesis of a precursor of auxin, tryptophan (Taiz and Zeiger, 1994). Hence, the changes in Zn status of the plant may modify hormonal balances in the plant, thereby affecting the growth process. Zinc is also a cofactor of many vital enzymes involved in the metabolism of bio-molecules (Ahmed *et al.*, 2012). Therefore, its supplementation may improve plant growth and development. The enhancing effects of vermicompost on the various growth-related characteristics have been recorded by some other researchers (Bachman and Metzger, 2008; Singh *et al.*, 2008; Singh *et al.*, 2010; Wang *et al.*, 2010; Warman and AngLopez, 2010; Mousavi and Ardebili, 2014) which are in agreement with the findings of the present study. The improvements in nutritional status in combination with the presence of humic acid as an effective enhancer factor as well as vermicompost-modified microbial population may be responsible for improving growth-related characteristics.

Except for individual application of ZnSO₄, the other applied treatments, especially the combined ones, resulted in significant increases in the contents of leaf proline in comparison to control (Table 4). In root tissues, vermicompost supplementation was the most effective factor affecting proline tissues and the application of Nano Zn or ZnSO₄ enhanced the rising impact of vermicompost on proline contents in combined treatments (Table 4). As it was shown in Table 5, leaf soluble phenols were significantly affected by the applied treatments where the highest amounts were found in Nano Zn-V. However, in root tissues, considerable increases were only caused by vermicompost treatment (Table 5). The results clearly indicated that the foliar applications of zinc, especially in nano-chelated form, is more effective on the physiological status of the leaves (the abovementioned parameters), whereas vermicompost influences root rather than shoot tissues. The modified contents of soluble phenolics and proline contents could be attributed to the alterations in the activities of some enzymes and modification in hormonal balances triggered by the applied supplementations.

Table 4. The effects of different applied nutritional treatments on the leaf and root proline contents

	Leaf proline content ($\mu\text{g g}^{-1}$ F.W.)	Root proline contents ($\mu\text{g g}^{-1}$ F.W.)
Control	82.59 ^{d**}	63.36 ^c
V*	113.33 ^c	102.5 ^b
Nano Zn	124.84 ^b	72.30 ^c
ZnSO ₄	93.78 ^d	64.63 ^c
Nano Zn-V	154.24 ^a	115.3 ^a
ZnSO ₄ -V	151.61 ^a	112.99 ^a

*: V; vermicompost

** : Data are means of three replicates. Mean values followed by different letter(s) are significantly different according to the Duncan's test.

Table 5. The effects of different applied nutritional treatments on the leaf and root soluble phenols

	Leaf soluble phenols (mg g ⁻¹ F.W.)	Root soluble phenols (mg g ⁻¹ F.W.)
Control	3.04e**	1.51 ^b
V*	7.31 ^c	2.19 ^a
Nano Zn	8.99 ^b	1.63 ^b
ZnSO ₄	3.74 ^d	1.62 ^b
Nano Zn-V	10.09 ^a	2.32 ^a
ZnSO ₄ -V	9.41 ^b	2.25 ^a

*: V; vermicompost

** : Data are means of three replicates. Mean values followed by different letter(s) are significantly different according to the Duncan's test.

Zn is known as a critical micronutrient and its implications in different aspects of cell metabolism like protein synthesis and nucleic acid metabolism have been well documented (Taiz and Zeiger, 1994; Ahmed *et al.*, 2012). It has been shown that Zn as a cofactor of many critical enzymes may modify many metabolism-related processes such as photosynthesis and biosynthesis of various biomolecules, especially nucleic acids, protein and carbohydrate (Ahmed *et al.*, 2012). Zn is an essential oligo-nutrient for the synthesis of tryptophan, a precursor of auxin and an important plant hormone (Taiz and Zeiger, 1994). It has been reported that the deficiency of Zn affects the levels of gibberellic acid in *Zea mays* L. (Sekimoto *et al.*, 1997). It is obvious that auxin regulates and controls many crucial processes related to the growth, development, and metabolism (Shahab *et al.*, 2009). It has been stated that vermicompost may influence various aspects of plant physiology and biochemistry (Sahni *et al.*, 2008; Ladan Moghadam *et al.*, 2012). Vermicompost supplementations led to significant increases in the contents of ascorbic acid, phenols, and flavonoids (Wang *et al.*, 2010). The favorable promoting effects of vermicompost utilizations on the various qualitative and quantitative characteristics in plants have been attributed to the presence of humic substances which have hormone-like activity (Atiyeh *et al.*, 2002) and enhance plant nutrition (Sahni *et al.*, 2008). In addition, vermicompost modifies the physical and biological characteristics of the soil (Srivastava *et al.*, 2012) and availability of macro and micronutrients (Sahni *et al.*, 2008).

In conclusion, this research shed light on the efficiencies of different kinds of fertilizer on the growth and physiology of *Chrysanthemum morifolium* as an important ornamental plant. It seems that the simultaneous supplementations of soil with the biological fertilizers and foliar applications of nano-fertilizers may be regarded as a suitable eco-friendly way to improve plant metabolism and growth.

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