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Morphophysiological Response of *Gynura aurantiaca* to Application of Humic Acid and Vermicompost

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Abstract

This study investigated the effect of humic acid and vermicompost as natural soil fertilizers on morphophysiological characteristics in *Gynura aurantiaca*. To determine the effect of these substances at different doses on the growth and antioxidant enzyme activity of *Gynura*, vermicompst (0, 2, 4 and 6%) and humic acid (0, 0.25, 0.5 and 1 g/kg) were applied at soil applications. Morphological characteristics of *G. aurantiaca* including stem length, leaf area, stem diameter, leaf diameter, the weight of fresh and dry roots and shoots and its physiological and biochemical characteristics including the content of chlorophyll a, b and total, soluble solids, and antioxidant enzymes activity were studied. The experiment was conducted as a factorial experiment based on completely randomized design with three replications in the research greenhouse of Shiraz University in 2019. Treated plants with 0.5 g/kg humic acid in combination with 6% vermicompost increased the root dry weight. 1 g/kg humic acid in co-application with 6% vermicompost enhanced leaf area, stem diameter, leaf diameter, plant height, root fresh weight, shoot fresh weight and shoot dry weight as well as the antioxidant enzymes activity of POD and CAT. The improved chlorophyll a, b and total contents were observed in plants treated with 0.5 g/kg humic acid in combination 4 % vermicompost. These results indicate that co application of humic acid and vermicompost is a useful fertilizer to improve growth in *Gynura* plants.

Keywords: Antioxidant enzyme activity, Biofertilizer, Chlorophyll content, Sustainable agriculture.

INTRODUCTION

A substantial increase was found in the use of organic additions in agriculture in recent years, owing to rising concerns about environmental issues such as soil degradation, waste recycling, as well as societal demands for more ecologically friendly plant and food production systems. This has helped to enhance the scientific research about the effects of these amendments on both the soil and the plant. Although, the majority of this study has concentrated on the effects of well-known organic additions such as animal manures, green manures, and compost, research into further alternative organic amendments such as vermicompost and humic acid is becoming increasingly important (Edwards *et al*., 2004; Lazcano and Dominguez, 2010).

Humic acid, a natural polymer with carboxyl and phenolic positions for the exchange process, is a blackish or brownish- colored. Humic acid constitutes stable and soluble complexes with micronutrients as a result of chemical and biological decomposition of soil organic matter, pit, lignin, and other materials. Additionally, it has a molecular weight of 3,000 to 30,000 daltons (Befrozfar *et al*., 2013; Boogar *et al*., 2014; Noroozisharaf and Kaviani, 2018; Karimi *et al*., 2020). Humic acid has been shown to have a direct impact on plant growth components like cell permeability, respiration, photosynthesis, and cell elongation. Other effects of humic acid on decreasing the incidence of plant disease have been demonstrated in numerous researches. Aside from the obvious effects on nutrient absorption and plant primary metabolism, humic chemicals may also have a significant impact on secondary metabolism (Noroozisharaf and Kaviani, 2018). Early flowering (Baldotto and Baldotto, 2013), increased leaf area (Ahmad *et al.*, 2013), number of flower and leaf, plant height, flower dry weight (Mirzaee Esgandian *et al*., 2020), chlorophyll content (Haghighi *et al*., 2012), and macro and micronutrient acquisition were all stimulated by the application of humic acid (Karimi *et al*., 2020).

Vermicomposts as a product of nonthermophilic bio-degradation of organic materials, are the result of interactions between an active biological mixture of bacteria, enzymes, plant residue, manure, and earthworm cysts (capsules) (Lazcano and Martin, 2010; Befrozfar *et al*., 2013; Sardoei *et al*., 2014). Vermicompost improves soil aeration and structure, reducing soil compaction and enhancing water retention potential, through its high organic matter content. By promoting improved root growth, nutrient absorption, and soil nutrient status, it also enhances soil nutrient status, both macro- and micro-nutrients (Lazcano and Dominguez, 2011). These materials have been found to show the same impacts with growth regulators and hormones (Mahboub Khomami *et al*., 2021).

Previous greenhouse and field researches demonstrated the impacts of numerous vermicomposts on ornamental foliage plants and cut flowers (Atiyeh *et al*., 2000; Mahboub Khomami, 2011). Significantly, hastening plant growths after substitution of vermicompost in growth medium, owing to modifications of water usability, enhancing access to micro and macro nutrients, provoking bacterial activity, increasing the activity of particular enzymes, making growth accelerating materials followed from the interaction of microorganisms and earthworms (Mahboub Khomami *et al*., 2021). Application of 50% of vermicompost fertilizer improved cranesbill growth and flowering attributes (Borji *et al*., 2014). The use of vermicompost made from water hyacinth (*Eichhornia crassipes*) improved the development and flowering of *Crossandra udulaefolia* substantially (Gajalakshmi and Abbasi, 2002). Vermicompost has been found to have a higher proportion of nitrogen, carbon, and mineral resources that is suitable for the need of the recipient plant. The use of such nutrients provides resources necessary for the formation of molecules in plants, resulting in improved growth, increased disease resistance, and the ability to combat unpleasant chemical substances found in the proximity of the plants. Various examples of such plant action have been mentioned (Sardoei *et al*., 2014).

The genus *Gynura* Cass., which belongs to the Asteraceae family, has more than 50 species of perennial herbs and subshrubs native to tropical Africa and Asia (Huxley *et al*., 1992). The leaves of *G. aurantiaca* (Blume) Sch. Bip. ex DC. are covered in numerous tiny, vivid purple hairs that give the plant a rich, colourful glow (Chen *et al*., 2002). So, it is well known as an ornamental foliage plant. Purple passion, velvet plant, or purple velvet plant are all common names for *G. aurantiaca*. As purple passion is young, it has an upright habit, but as it become mature, it shows more vine-like and spreading habit, which make it ideal for hanging baskets. Furthermore, the plant's distinctive coloration makes it ideal for adding a splash of color to combination planters. The rich purple color is really appealing (Cui *et al*., 2019).

The application of the organic fertilizers is one of the suitable methods in creating plants, owing to the remarkable impact on advancing soil properties, decreasing ecological side effects, and better plant growth; nonetheless, information on the effects of humic acid and vermicompost on plant growth and physico-biochemical attributes of *Gynura aurantiaca* is lacking and inconclusive. The slow growth of *Gynura* plants during growth and development is one of the most important problem these days. It was thus decided to investigate the effects of using organic fertilizers (humic acid and vermicompost) instead of chemical fertilizers on improving the ornamental characteristics of *Gynura aurantiaca*, such as leaf area, antioxidant enzyme activity, total carbohydrate content, and chlorophyll content.

MATERIALS AND METHODS

single-leaf stem cuttings of *Gynura aurantiaca* were purchased from Armaghan Plant Company, Shiraz, Iran. Cuttings were cultivated in moist perlite culture medium and harvested after rooting completely (20 days after planting) in 2019 at the greenhouse with average day/night temperature was 28 ± 2/20 ± 2 °C with relative humidity of 55±5%.

In order to keep the humidity around the cuttings, the culture medium was covered with transparent plastic. However, the plastic was removed for one hour every day to prevent stalk rot. The rooted plants were sown in pots with the rim diameter of 7 cm and filled with perlite: peat moss: leaf mold (1:1:1).

This research was conducted as a factorial experiment based on a completely randomized design (CRD) with three replications. As the plants reached 3-4 fully expanded leaf stage, they were transferred to the larger pots (diameter of 10 cm) and filled with sand: peat moss: leaf mold (2:1:1).

After a week, humic acid (Huminbest 85%, Germany) (0, 0.25, 0.5, 1 g/kg) and vermicompost (0, 2, 4, 6 %) were added in to the cultivation media. The treatments were applied monthly, and continued for three months. The following attributes were measured, one month after the last treatment.

Plant length and stem diameter

At the end of the experiment, the stem length and diameter were measured by ruler a digital caliper, respectively.

Leaf area

At the end of the experiment, leaf area was measured (Delta-T-Devices LTD England) in 3 leaves. The results were expressed as a mean value of three leaves.

Leaf diameter

At the end of the experiment, leaf diameter was measured using digital caliper.

Fresh and dry weight of shoots and roots

At the end of the experiment, roots and shoots were measured using a digital balance with 0.001 g accuracy. To measure the dry weight of roots and shoots, these organs were placed in separate envelopes and dried in an oven at 60 °C for 48 h and then it was measured by a digital balance.

Chlorophyll contents a, b, and total

Chlorophyll (chlorophyll a, chlorophyll b and total chlorophyll,) contents of the leaf samples were measured following the formula described by Arnon (1949), as DMSO is used as the extraction solvent. The optical density (OD) of the extract solution was recorded at 663 and 645 nm by the spectrophotometer (Epoch, Bio Tek©, USA). The final content of each pigment was expressed as mg per g fresh weight of the leaves.

1) Chlorophyll a (mg/g FW) = (12.7 (A_{663}) - 2.69 (A_{645}) × Volume made)/(Wt of the sample)
2) Chlorophyll b (mg/g FW) = (22.9 (A_{645}) - 4.68 (A_{663}) × Volume made)/(Wt of the sample)
3) Total Chlorophyll (mg/g FW) = (20.2 (A_{645}) + 8.02 (A_{663}) × Volume made)/(Wt of the sample)

where Wt is the weight of the sample and A λ is the absorption at wavelength λ (nm).

Soluble solids content

Soluble solids content was extracted twice from 0.1 g of leaf powder with ethanol (80%) and estimated by using phenol reagent, according to the protocol described by Fox and Robyt (1991). The absorbance of the samples was determined at 490 nm, using a spectrophotometer.

Antioxidant enzymes activity

The enzyme extraction of leaf samples was prepared according to the protocol described by Ozden *et al.* (2009). To determine the CAT enzyme activity, 1 mL of the reaction mixture (50 mM phosphate buffer (pH 7.0), 15 mM of H_2O_2 and 50 µl of enzyme extract) was measured at 240 nm. CAT enzyme activity was determined as the reduction of H_2O_2 with the extinction coefficient of 39.4 mM^{-1} cm^{-1}. The activity of SOD enzyme was measured at 560 nm using the method described by Giannopolitis and Ries (1977) and expressed as unit/mg FW. For POD assay, the reaction (50 µl of enzyme extract, 2.9 ml of 10 mM potassium phosphate buffer (pH = 7) and 0.05 ml of 20 mM guaiacol) was started by adding 20 µL of 40 mM H_2O_2 and the activity of the enzyme was determined using the extinction coefficient of 26.6-1 mM^{-1} cm^{-1}.

Statistical analysis

This research was conducted as a factorial experiment based on a completely randomized design (CRD) with three replications data were analyzed statistically using SAS software (ver. 9.4) and means were compared using the least significant difference (LSD) test at P < 5%.

RESULTS

Root and shoot growth

Application of 1 g/kg humic acid increased the leaf area (128.67%), stem diameter (12.63 %), leaf diameter (13.63%), plant height (88.36 %), shoot fresh weight (176.84 g), root fresh weight (276.76 %), shoot dry weight (127.58) and root dry weight (143.47%) of *Gynura* plant, compared with the control (Fig. 1). When humic acid was used in combination with vermicompost, the shoot and root growth was nearly doubled. The 0.5 g/kg humic acid in combination with 6% vermicompost increased the root dry weight by 330 %. Treatment of plants by 1 g/kg humic acid in combination with 6% vermicompost increased the leaf area, stem diameter, leaf diameter, plant height, root fresh weight, shoot fresh weight and shoot dry weight by 415 %, 37.90 %, and 65.90 %, 269.98%, 404.57, 282.17% and 400%, respectively (Fig. 1).

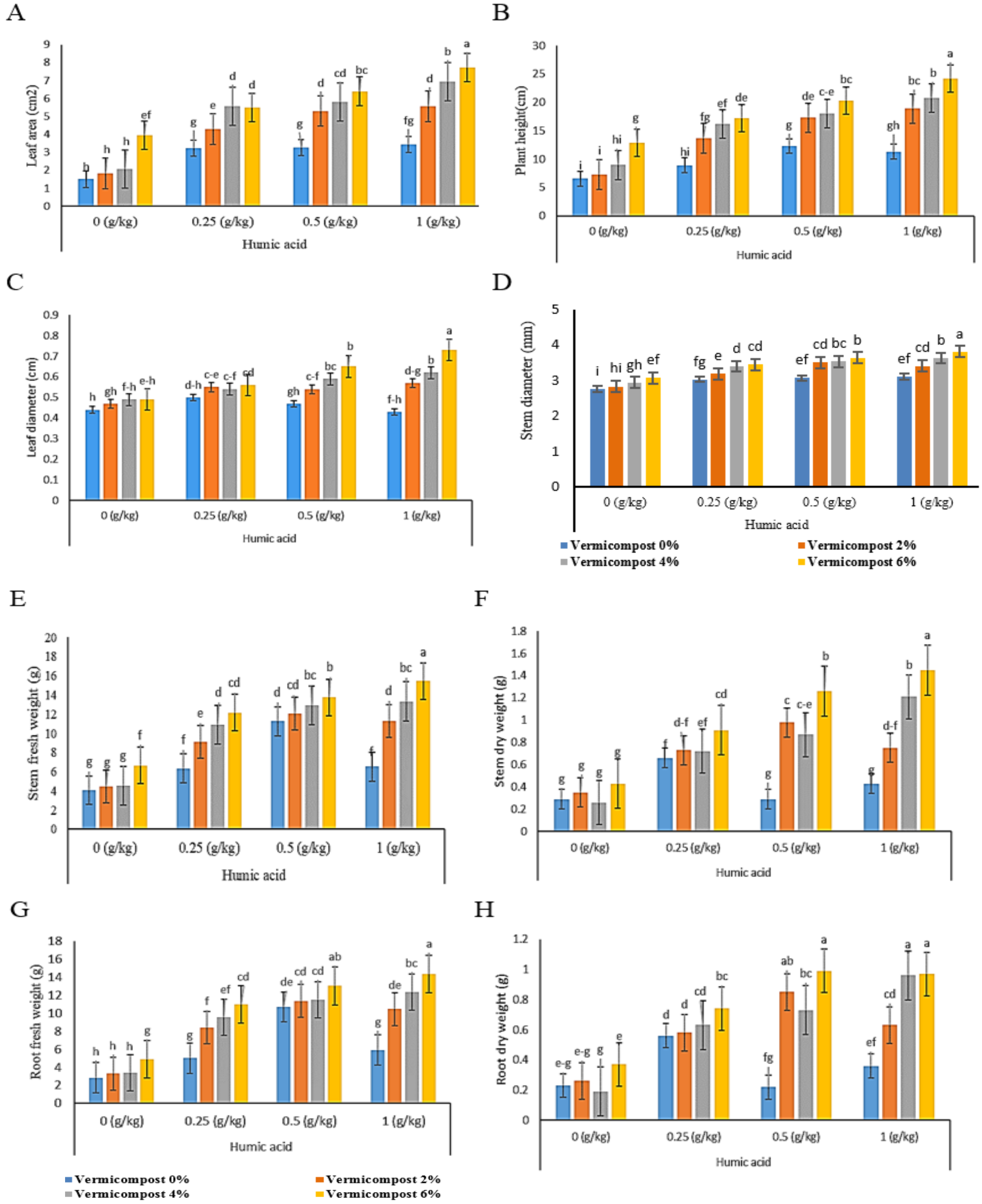

Fig. 1. The effect of humic acid and vermicompost on leaf area and diameter, plant height, stem diameter, fresh and dry weight, and Root fresh and dry weight.

Chlorophyll content and total soluble solids

Fertilization of *Gynura* plants with 1 g/kg humic acid doubled the chlorophyll a, b and total contents of leaves, compared with the control (Fig. 2). When humic acid was used in combination with vermicompost, the chlorophyll content was nearly tripled. The 0.5 g/kg humic acid in combination 4 % vermicompost improved the chlorophyll a, b and total contents by 3-fold amount (Fig. 2).

1 g/kg Humic acid increased the total soluble solids (147.36 %) of *Gynura* leaves, compared with the control (Fig. 2). When humic acid was used in combination with vermicompost, the total soluble solids content was greatly increased. Co-application of 1 g/kg humic acid with 4% vermicompost amplified the amount of total soluble solids contents by 236.84 % (Fig. 2).

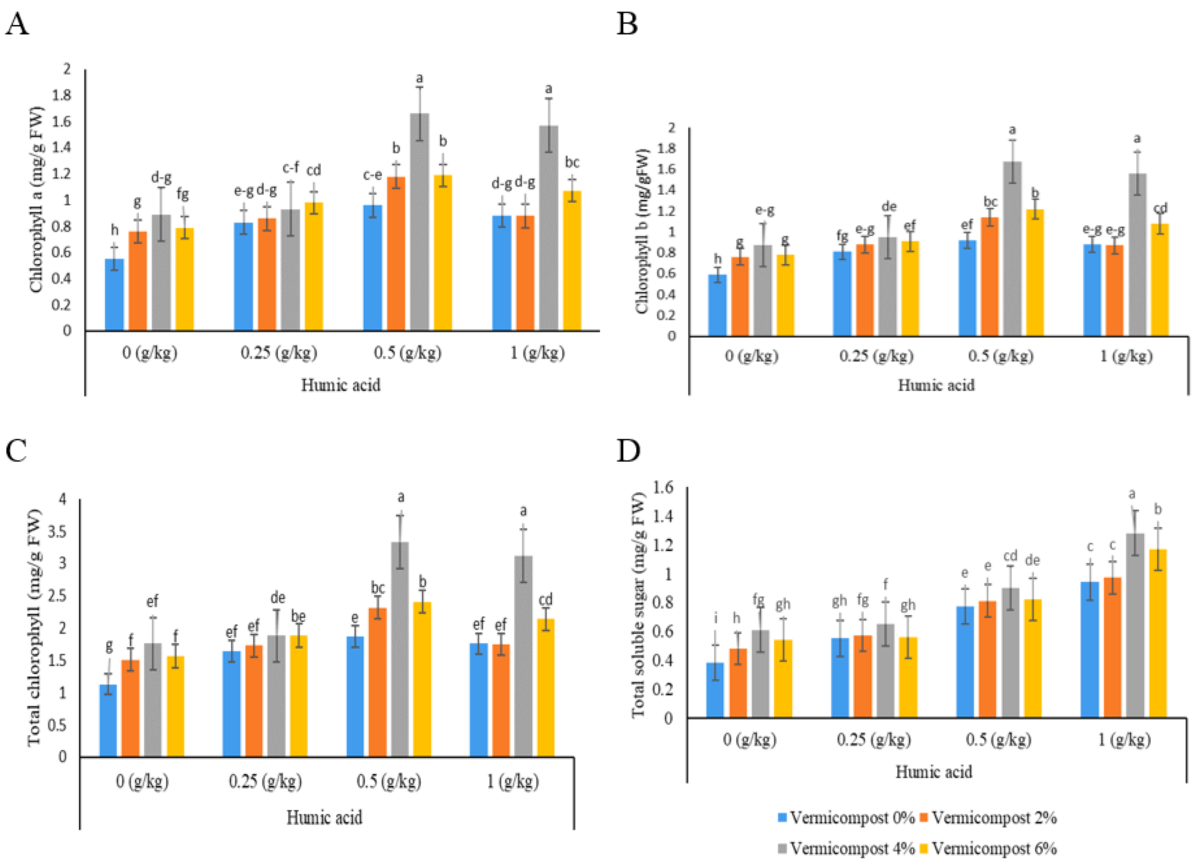

Fig. 2. The effect of humic acid and vermicompost on chlorophyll (a, b and total) and total soluble solids content of *Gynura* plants.

Antioxidant enzymes activity

1 g/kg Humic acid increased the antioxidant enzymes activity of SOD (155.75 %), POD (620 %) and CAT (200 %) of *Gynura* plants, compared with the control (Fig. 3). When humic acid was used in combination with vermicompost, the antioxidant enzymes activity was strongly increased. The 1 g/kg humic acid in co-application with 6% vermicompost increased the antioxidant enzymes activity of POD and CAT by 920 % and 345.83 % respectively (Fig. 3). Treatment of plants by 1 g/kg humic acid in combination with 4% vermicompost increased the antioxidant enzymes activity of SOD by 228.25%, having no significant difference with 1 g/kg humic acid in combination with 6% vermicompost.

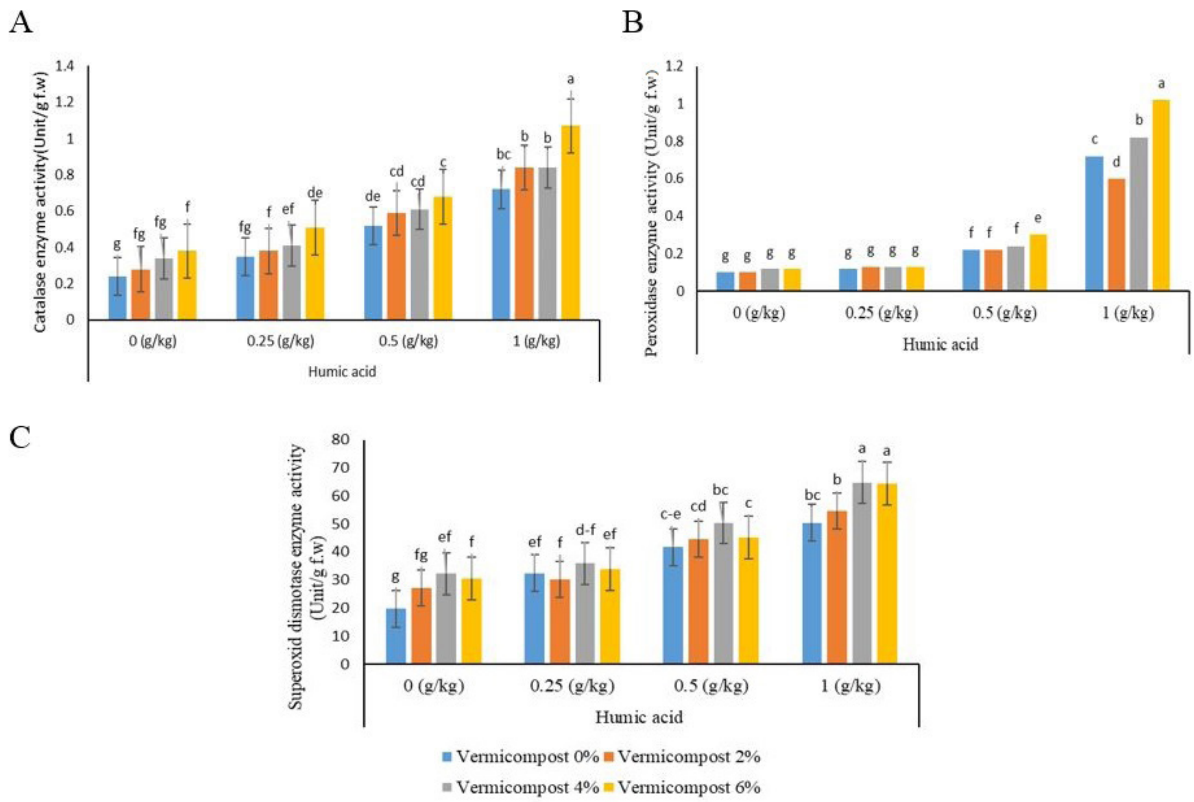

Fig. 3. Effect of different levels of vermicompost and humic acid on antioxidant enzymes activity of *Gynura* plants.

DISCUSSION

The current study showed that higher concentrations of humic acid and vermicompost improved root growth (fresh and dry weight), which was in accordance with Nikbakht *et al.* (2008), Boogar *et al.* (2014), and Joshi *et al*. (2015) findings. Root development is a result of enhanced nutrient absorption in the root, as well as the hormone-like effects of humic acid. Humic acid enhanced the number of lateral roots of gerbera flowers due to its increased nutrient intake and hormone-like characteristics (Boogar *et al.*, 2014). Vermicompost's hormone-like activity leads to an increase in root biomass, root initiation, and improved plant growth and development (Joshi *et al.,* 2015). Previous studies have shown that humic acid leading to support photosynthetic tissues, resulted in an increased total dry weight (Motaghi and Nejad., 2014). Water soluble bioactive compounds including phytohormones, humic, and fulvic acids, minerals, amino acids, or microbial metabolites found in vermicompost may stimulate the plant growth (shoot length, shoot diameter, leaf area and leaf diameter) (Chaichi *et al.*, 2018). It has been reported that vermicompost not only increases the availability of mineral elements required by plants, but also promotes optimal growth and nutrient availability by increasing the physical condition and functions of microorganisms (Anwar *et al.*, 2005). The increase in plant growth due to the mature vermicompost consumption can be attributed to its content of physiologically active plant growth affecting chemicals as well as nutritional factors (Hatamzadeh, 2011; Moghadam *et al.,* 2012). As perceived in this study, humic compounds have the largest impact on root nourishment, which leads to higher shoot development and shoot dry weight (Chaichi *et al.*, 2018). Given to the effects of humic acid and vermicompost on *Gynura* growth, one of the reasons for the increase of leaf area is the role of these elements in increasing photosynthetic activity, which leads to the increased leaf area (Moghadam *et al.*, 2012; Ahmad *et al.*, 2013).

Based on results of this study, high content of chlorophyll resulted in high photosynthesis rate, which in turn high carbohydrate content should be respected. The chlorophyll content and contents of total soluble solids in the leaves of *Gynura* increased significantly after being treated with 1g/kg humic acid and 4% vermicompost fertilizers compared with those of the control. In previous study, humic acid fertilizer increased chlorophyll content and photosynthesis, resulting in an increased carbohydrate stimulation in cut chrysanthemum flowers, which had a direct impact on floral quality (Fan *et al.*, 2015). Furthermore, previously, the positive effect of vermicompost on higher content in carbohydrates and total soluble solids has been reported (Canellas *et al.,* 2015; Kim *et al.,* 2015). An increase in leaf chlorophyll concentration as a result of vermicompost treatment can be interpreted as a sign of better plant physiological health (Ievinsh *et al.,* 2017). This impact was found in both applications of humic and vermicompost in the current study. Moreover, many authors supported findings (Ahmad *et al*., 2013; Tina *et al.,* 2015; Ievinsh *et al*., 2017), implying that vermicompost components activate photosynthesis-related process.

SOD is the initial line of defense against superoxide anion radicals, converting them to O_2 and H_2O_2; CAT and APX then detoxify H_2O_2 to H_2O and O_2 due to peroxide's high cell integrity toxicity (Morozesk *et al*., 2017). The findings of this study showed that humic acid in combination with vermicompost stimulated SOD, POD, and CAT activity in *Gynura*, thereby enhancing plant antioxidant system and showing improvement in growth and development of these plants, which was in accordance with Garcia *et al.* (2016) findings. There has been observed the effect of application of vermicompost and humic acid in inducing antioxidant enzyme activity of aerial sections of *Tulbaghia ludwigiana* (Aremu *et al*., 2014) and rice (Hernández *et al*., 2012; Berbara and Garcia, 2014).

CONCLUSION

Our findings could advance the understanding of physiochemical responses and antioxidant enzyme activity in *Gynura* plants treated with soil application of humic acid and vermicompost. The present study demonstrated humic acid and vermicompost applications enhanced vegetative growth (leaf area, plant height, stem and leaf diameter) in *Gynura* plants. The increased antioxidant enzyme activity after humic acid and vermicompost applications evidenced the effects of these compounds on enhancing plant antioxidant systems. Thus, co application of humic acid and vermicompost increased the total soluble solids of *Gynura* plants. In general, the application of these substances can be aid to improve sustainable agriculture systems around the world, typically in areas were the application of are an economically restrictive factor.

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