Morpho-physiological traits and macro-elements content of pansy (Viola tricolor L.) affected by foliar application of bio-stimulants

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Abstract

Pansy (Viola tricolor L.) is from the Violaceae family that is planted in the gardens as a ground cover for early spring color and medicinal herb. This experiment was carried out to evaluate the effects of bio-stimulant compounds on the growth traits and macro-elements content of pansy. This experiment was conducted on the basis of randomized complete blocks design (RCBD) with nine treatments and three replications. The treatments included the commercial formulations of aminolforte, kadostim, fosnutren, humiforte (each of them at 2 and 4 L.ha⁻¹), and control treatment. The effects of treatments were significant (p≤0.01) on all of the traits except for the plant height, collar diameter of stem and total dry weight. The greatest amount of the stem diameter (5.01 mm) and number of leaves (44 leaves.plant⁻¹) in 2 L.ha⁻¹ aminolforte treatment, calyx and flower diameter (7.25 and 48.22 mm) in the treated plants by 2 L.ha⁻¹ fosnutren was obtained. The highest number of flowers (26 flowers.plant⁻¹) in 2 L.ha⁻¹ humiforte and total fresh weight (34.90 g.plant⁻¹) in the treatment of 2 L.ha⁻¹ kadostim was observed. The maximum amount of SPAD value (49.81 SPAD) with 2 L.ha⁻¹ humiforte, nitrogen (3.44%) in the foliar application of 4 L.ha⁻¹ aminolforte, phosphorous (0.28%) in 4 L.ha⁻¹ fosnutren and potassium (4.38%) in 4 L.ha⁻¹ humiforte was achieved. The least amount of all traits was recorded in control treatments. The growth traits and macro-elements content of pansy plants increased due to foliar application of bio-stimulants compounds.

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**Introduction**

Pansy (*Viola tricolor* L.) is from the Violaceae family. There are approximately 500 species of pansy of which most are low growing herbaceous annuals or perennials. Pansy is widely distributed across northern and southern temperature zones. Pansy is native to a broad area of Europe, Africa, and Asia. *V. tricolor* L. is European and has become naturalized in North America. Flowers have various color combinations of yellow, purple-red, violet-blue, or white. Pansy is planted in the gardens as a ground cover for early spring color and is treated as perennials (Dole and Wilkins, 1999). The plants, especially the flowers, contain antioxidants and are edible. Extracts from the plant are anti-microbial. Many cyclotides, found in pansy are cytotoxic. This feature means that it could be used to treat cancers. Additionally, the medicinal properties of pansy are mucilaginous, laxative, depurative, expectorant, emetic, alterative, anti-inflammatory, diuretic, sedative, antioxidant as well as antiseptic (Tang et al., 2010).

Amino acids are the essential constituents in the all plant cells. In addition to their role in protein synthesis, they participate in both primary and secondary metabolic processes associated with plant development in responses to stress. So that, glutamine, glutamate, aspartate, and asparagines serve as pools and transport forms of nitrogen, as well as in balancing the carbon/nitrogen ratio. Other amino acids such as tryptophan, methionine, proline and arginine contribute to the tolerance of plants against biotic and abiotic stresses either directly or indirectly by serving as precursors to secondary compounds and hormones (Muller and Touraine, 1992). Micro and macro elements existing in bio-stimulants formulations play an important role in promoting the growth and production of plants. Micro elements participate in most of the enzymatic reactions and they also play a role indirectly through the synthesis of several growth regulators. The beneficial effects of these elements were reported by several authors, such as Naghuiub et al. (2005), and Soleymani and Shahrajabian (2012), spraying Zn, Mn, Cu, and Fe on pot marigold (*Calendula officinalis* L.) and sorghum (*Sorghum bicolor* (L.) Moench) plants. Due to the high economic, ornamental, and medicinal value of the pansy, and the many uses of the plant bio-stimulants as a new technique in the plant productions, we need to recognize the productive viability of the use of bio-stimulants especially based on amino acids. The aim of this study was to investigate the effects of bio-stimulants on the morpho-physiological traits and macro-elements content of *Viola tricolor* L.

**Material and methods**

*Plant material and location*

This experiment was carried out in 2014 at the Institute of Medicinal Plants (35° 54´ N and 50° 53´ E; 1500 m elevation) in the Academic Center for Education, Culture and Research (ACECR). The soil was loam-silt with 0.071% nitrogen, 8.4 p.p.m phosphorous, 163.4 p.p.m potassium, 2.71 ds.m⁻¹ EC, and 8.3 pH. The seeds of *Viola tricolor* L. with proper quality of germination were supplied from the seed bank of the Institute of Medicinal Plants (IMP). The suitable seedlings selected with similar height and weight. Other operations were done regularly during the growing season.

*Treatments*

This study was conducted on the basis of a randomized complete blocks design with nine treatments and three replications. The treatments were control (foliar spray with distilled water) and bio-stimulants with commercial formulations of aminolforte (A₁ and A₂), kadostim (K₁ and K₂), fosnutren (F₁ and F₂) and humiforte (H₁ and H₂) each of them applied at 2 and 4 L.ha⁻¹, respectively. Four commercial formulations of bio-stimulants including humiforte, aminolforte, kadostim and fosnutren were supplied by Inagrosa Industries Agro-Biologicals (Anonymous, 2014). The details of the formulations are shown in Table 1.

*Foliar application*

To increase the absorption of solutions by plants, the foliar application of bio-stimulants was done in
conditions without wind and rain, and before sunrise when the plant stomata are open (Sergiand Leonor, 2002). Foliar application on the shoot of pansy was done in four times during growth stages with eight days intervals. The first spray was applied three days after transplant and the others were applied 12, 21 and 30 days (flowering stage) after transplant.

Evaluation of traits
Samples in nylon bags were sent to the laboratory for measuring traits. The studied traits were plant height (cm), collar diameter of stem (mm), stem diameter (mm), number of leaves (leaves.plant⁻¹), calyx diameter (mm), flower diameter (mm), number of flowers (flowers.plant⁻¹), total dry weight (g.plant⁻¹), total fresh weight (g.plant⁻¹), SPAD value (SPAD), nitrogen (%), phosphorous (%) and potassium content (%). For the measurement of leaf SPAD value, five leaves of each plant were selected and mean of the leaf SPAD value was measured by a device for SPAD (Minolta, 50 II). Total Nitrogen, phosphorus, and potassium were determined in the dried leaves according to Antil et al. (2009).

Statistical analysis
An experiment was laid out in a randomized complete block design (RCBD) with nine treatments and three replications. Analysis of variance of the results was done using by the SPSS software (ver.17), and means in the results were compared using by the Duncan’s Multiple Range Test at a probability level of 0.05.

Results
Morpho-physiological traits
According to the results of analysis of variance, the effect of bio-stimulants on the stem diameter, number of leaves per plant, calyx and flower diameter, number of flowers per plant, total fresh weight, and SPAD value was statistically significant (P≤0.01), while it was insignificant for the plant height, stem diameter, and total dry weight (Table 2). The maximum height of the plant and total dry weight was obtained in the treatment of 2 L.ha⁻¹ kadostim. The greatest collar diameter of stem and total fresh weight in 4 L.ha⁻¹ aminolforte, stem diameter in 2 L.ha⁻¹ fosnutren, flower diameter in treated plants with 2 L.ha⁻¹ aminolforte, and SPAD value (49.81 SPAD) in the treatment of 2 L.ha⁻¹ humiforte was observed (Table 3).

Table 1. Formulation of bio-stimulants used in the experimental treatments.

<table>
<thead>
<tr>
<th>Bio-stimulants*</th>
<th>Formulation of compounds**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminolforte</td>
<td>3750 mg.L⁻¹ free amino acids, 2% organic components, and 1.1% total N (0.8% N of urea and 0.3% organic N)</td>
</tr>
<tr>
<td>Kadostim</td>
<td>3750 mg.L⁻¹ free amino acids, 2% organic components, and 5% total N (1.6% N of amonia, 3.1% N of nitric and 0.3% organic N) and potassium 6% K₂O</td>
</tr>
<tr>
<td>Humiforte</td>
<td>3750 mg.L⁻¹ free amino acids, 2% organic components, and 6% total N (1.3% N of amonia, 3.7% N of urea, 0.5% N of nitric, and 0.3% organic N), potassium 5% K₂O, and phosphorous 3% P₂O₅</td>
</tr>
<tr>
<td>Fosnutren</td>
<td>3750 mg.L⁻¹ free amino acids, 2% organic components, and 3.8% total N (2.1% N of amonia, 1.4% N of nitric, and 0.3% organic N), and phosphorous 6% P₂O₅</td>
</tr>
</tbody>
</table>

*Bio-stimulants supplied by Inagrosa Industries Agro Biologicals are compatible to the climate of Iran.
**Quantity and kind of free amino acids applied in the formulation of bio-stimulants in this experiment based on the percent of total amino acids are as follows: 11.2% Gylsin, 5.1% Valine, 8.3% Proline, 13.2% Alalin, 4.4% Aspartic acid, 8.3% Arginine, 0.9% Glutamic acid, 5.1% Lysine, 16.4% Lucine, 4.4% Isolucine, 5.1% Phenylalanin, 4.2% Methionine, 3.9% Serin, 0.3% Treonine, 0.3% Histidine, 1.5% Tyrosine, 0.9% Glutamine, 0.3% Systein, 0.4% Aspargine, and 0.4% Tryptophon.

Macro-elements content
The results of the analysis of variance showed a significant effect (P≤0.01) of bio-stimulants on the nitrogen, phosphorous and potassium content (Table 2). In relation to the mean comparisons, the highest content of nitrogen (3.44%) in the treated plants by 4 L.ha⁻¹ aminolforte, phosphorous (0.28%) in the treatment of 4 L.ha⁻¹ fosnutren, and potassium (4.38%) in the treatment of 4 L.ha⁻¹ humiforte was achieved. On the other hand, the least amount of all traits was observed in control treatment (Table 3).
Discussion and conclusion

The foliar application of bio-stimulants appeared to have a significant and positive effect on the morpho-physiological and phytochemical traits of pansy plants. The stem diameter increased with the application of 2 L.ha⁻¹ aminolforte. The results of this study are in line with results of the experiments of Mazher et al., (2011) on Codiaeum variegatum L., and Farooqi et al. (1996) on Artemisia annua L. Nahed et al. (2010) concluded that all growth traits improved with an increase in concentration of amino acids. The positive effect of amino acids on plant growth traits might be due to the stimulating effect of amino acids on plant cells growth. Bio-stimulants increased leaves number to the highest amount in the treatment of 2 L.ha⁻¹ aminolforte. These results are in accordance with results of an experiment by Abou Dahab and Nahed (2006). They suggested that the use of amino acids (especially tryptophan) were significantly effective on the morphological traits of philodendron. However, amino acids were introduced by Goss (1973) as a source of energy when there is a lack of carbohydrates. The calyx and flower diameter increased with the application of amino acid compounds and the best treatment for these traits was the application of 2 L.ha⁻¹ fosnutren. These results are in accordance to Christopher et al. (2007) on Basella rubra L. An increase in yield and growth parameters is proven to be feasible by using amino acids. Therefore, a supply of nutritious sources to form protein tissue is essential (El-Tantawy, 2009). Amino acids are the precursor of polyamines which are essential in the regulation of plant growth and development (Martin-Tanguy, 2001). Also, the number of flowers per plant increased with the application of bio-stimulants especially treatment of 2 L.ha⁻¹ humiforte to the amount of 26 flowers. Amino acids with activate of the effective hormones in the flowering initiation and fruit set, improvement of pollen germination and increase in the rate of flowering cause improve in the pollination and flower number (Zhang et al., 2010). The yield of total fresh weight increased about 50.7% with application of 2 L.ha⁻¹ kadostim. The results showed that the treatment of kadostim were capable of increasing biomass, stem branches, and leaves in comparison with the control treatment. The above findings agree with the study done by Raeisi et al. (2014). In an
In this experiment, commercial formulation of bio-stimulants based on bio-active amino acid compounds accompanied by macro-nutrients of N, P and K had a positive effect on growth traits and macro-elements content of pansy plants.

**Acknowledgements**

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**References**


Christopher PA, Viswajith V, Prabha S, Sundhar K, Malliga P. 2007. Effect of coir pith based cyanobacterial basal and foliar bio-fertilizer on...
Nia et al. Int. J. Biosci. 2015


Raeisi M, Babaie Z, Palashi M. 2014. Effect of chemical fertilizers and bio-stimulators containing amino acid on quality and quantitative and qualitative characteristics of tomato (Lycopersicum esculentum)
http://dx.doi.org/10.12692/ijb/4.1.425-431


http://dx.doi.org/10.1104/p.125.2.1094

http://dx.doi.org/10.5539/ijb.v4p92

http://dx.doi.org/10.1002/hlca.201000115

http://dx.doi.org/10.1093/jxb/erq232