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Effect of biostimulant application on development of pansy (*Viola tricolor* var. *Hortensis* dc.) seedlings

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ABSTRACT

Pansy (*Viola tricolor* var. *hortensis* DC.) is a biennial large-flowered hybrid plant cultivated as a popular ornamental plant in urban green areas. The plants prefers sun and well-drained soil, but also tolerate low temperatures. The optimal time for transplanting pansy seedlings in outdoors beds is in autumn, although early autumn frosts can affect the insufficient nutrient uptake, which can be a limiting factor for seedling survival. To avoid these effects, plants can be treated with different growth stimulants such as the biostimulant Radifarm®. The aim of this study was to investigate the effects of biostimulant Radifarm® application on development of pansy seedlings. The experiments were carried out in the glasshouse of the Faculty of Agriculture in Banja Luka, Bosnia and Herzegovina, set up as a split-plot design with treated and untreated plants during 2014 and 2015. In the greenhouse, young plants were treated with biostimulant Radifarm® (Valagro SpA, Italy) in concentration of 0.30% while control plants were treated only with tap water. During the experiment, the following parameters were recorded: plant height (cm), plant diameter (cm), number of leaves, and number of flowers. At the end of the experiment root and above-ground fresh, and dry mass were recorded. Biostimulant treatment showed improved results by increasing the examined parameters. Biostimulant application can ensure production of high quality pansy seedlings by overcoming temperature stress and providing good nutrient uptake by the reduction of fertilizers and environmental contamination.

Keywords: abiotic stress, growth parameters, plant stimulants, seaweed extracts, transplants, pansy

INTRODUCTION

Pansy (*Viola tricolor* var. *hortensis* DC.) is a common ornamental plant in urban green areas. The English common names for this plant are "pansy", "viola", and "violet". The term "pansy" is reserved for multi-colored large-flowered hybrids that are grown for bedding purposes, while "viola" is usually reserved for smaller, more delicate plants. Modern horticulturists have developed a wide range of pansy flower colors including yellow, gold, orange, purple, violet, red, white, and even black (very dark purple). Pansies belong to perennials, but ornamentally are usually grown as biennials, developing only leaves in first year while the flowers and seeds are developing in the second year of growth. Usually they are grown in autumn as bedding plant, since they can survive light frosts and short periods of snow cover. In warmer climate zones, pansies can bloom over the winter and may re-seed themselves. However, they are not very heat-tolerant as high temperatures and warm air inhibit their blooming and in some cases can even cause wilting.

After transplanting the seedlings in outdoor beds, the plants are exposed to various types of environmental stresses. Some of them have genetic potential to resist or avoid negative stress, but sometimes it is impossible to avoid adverse circumstances that lead to stress (Bartwal et al., 2013). The most important abiotic stresses limiting agricultural productivity are drought, salinity, non-optimal temperatures, and low soil fertility (Bulgari et al., 2019). Reduction of symptoms caused by abiotic stresses is possible with different natural substances such as amino acids, humic acids, seaweed extracts which can be applied to plants, seeds or roots with the intention of stimulating natural processes of plants benefiting nutrient use, efficiency, and tolerance to abiotic stresses (Du Jardin, 2015). The aim of modern agriculture and floriculture is to reduce nutrient inputs without reducing the possible yield and quality of flower species (Bulgari et al., 2015). In order to overcome abiotic stresses that are caused by uneconomical application of fertilizers (which has harmful consequences to the environment by pollutes water and soil), we have to use new technologies such as biostimulants.

According to Bulgari et al. (2015), biostimulant application results in reducing fertilization requirements and thus aiding environmental conservation. Biostimulants applied in plant production have been widely considered as an environmental-friendly agricultural practice (Parađiković et al., 2019). Biostimulants are extracts obtained from organic raw materials containing components such as: seaweed extracts, mineral elements, humic and amino acids, vitamins, chitin, chitosan, and poly- and oligosaccharides (Hamza and Suggars, 2001 cited in Yakhin et al., 2017, Kauffman et al., 2007). Seaweed extracts have been used in agriculture as soil conditioners or as plant stimulators. They are applied as foliar sprays to enhance plant growth, photosynthetic activity and resistance to fungi, bacteria & viruses, as well as freezing, drought, and salt tolerance, and improving the yield quality and productivity of many crops (Sharmaet al., 2014). Silva et al. (2020) describes the positive effects of biostimulants based on seaweed extract, mainly green and brown algae, in combination with amino acids and chemical compounds such as potassium, phosphorus, nitrogen, boron, molybdenum and zinc on plant growth and quality, stress tolerance, nutrient accumulation, and biosynthesis. Humic acids produced by the decay of organic materials can be found in soil and peat and may stimulate plant growth, shoot elongation, the increase leaf nutrient accumulation and chlorophyll biosynthesis (Jindo et al., 2020). Parađiković et al. (2019) refers to many researches based on application of different biostimulants that improve root growth and development of many horticultural plants such as vegetables, ornamental and medical plants.

The aim of this study was to investigate the effects of biostimulant Radifarm® application on development of pansy seedlings.

MATERIAL AND METHODS

The experiments were carried out in the glasshouse of the Faculty of Agriculture in Banja Luka, Bosnia and Herzegovina during 2014 and 2015. Pansy (*Viola tricolor* var. *hortensis* DC.) seed and the substrate Fruhstorfer Erde, type Aussaat und Stecklinge (sowing substrate; Hawita Gruppe, Germany) were used in the experiment. The substrate contains perlite, peat and volcanic clay for better aeration and friability. Other substrate characteristics were: pH 5.9; N mg/L=80; P_2O_5 mg/L=60; K_2O mg/L=90; retention capacity 700 ml/L.

Seeds were sown on July 15th (in both years of investigation), in plastic propagation trays in sowing substrate. Plants emerged after 4 to 7 days and were kept in the same trays until seedlings had formed the third pair of true leaves. On the 5th of September, in each year, seedlings were transplanted into polyethylene pots, 9 cm diameter, in Baltisches substrat (planting substrate; Hawita Gruppe, Germany). According to the manufacturer, this substrate contains a mixture of clay, white peat, black peat, and perlite.

After transplanting, plants were arranged in a spilt-plot design in two groups marked as treatment and control, each containing four replications with 10 plants. The experiment had two factors: main factor was the treatment with biostimulant, and the second factor was the year of investigation (2014, 2015). Treatment group was treated with a 0.30% solution of biostimulant Radifarm® (Valagro SpA, Italy) applied to the plant rhizosphere. Control plants were treated only with tap water. The plants were treated with same rate of 200 mL per plant (biostimulant or water) every seventh day until 5th November. This is the period when plants could still be transplanted in outdoor beds. Composition of biostimulant applied is shown in Table 1.

At the end of the experiment (5th November), the plants were sampled to assess the effect of the biostimulant. The following parameters were recorded: plant height (cm), plant diameter (cm), number of leaves, flowers, fresh and dry weight of roots and above-ground plant parts. No additional fertilization or pesticide treatment were used in the experiments.

The average monthly temperatures for both years are present in Figure 1.

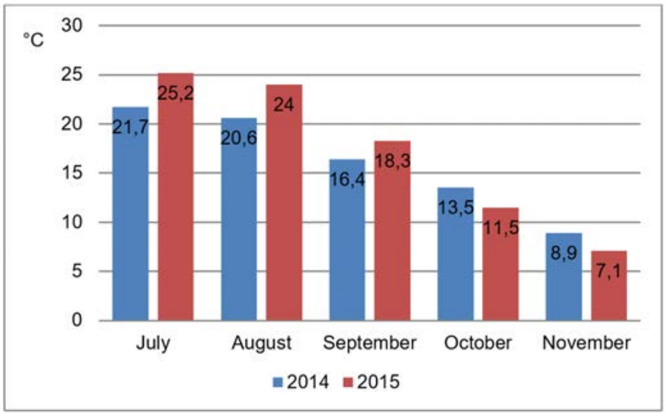

Figure 1. Average monthly temperature in 2014 and 2015 during the experiment (July-November)

Data was statistically analyzed using two-way analysis of variance ANOVA with two factors: the biostimulant treatment and a year of investigation. Fisher's LSD test was applied to judge statistical significance of differences between treatments using main values that were considered significantly different when $P<0.05$.

RESULTS AND DISCUSSIONS

The average values of several morphological parameters that have characterized the plant growth and development in two years of investigation are presented in the table 2. Morphological parameters such as plant height, plant diameter, number of leaves, and number of flowers were increased by biostimulant application. The biostimulant has positive effects on the growth parameters, and they can most likely be explained by their composition which consists of amino acids, humic acids, polysaccharides, and vitamin complex (Table 1).

According to Zeljković et al. (2013), the application of biostimulant Radifarm® stimulated plant growth, increased number of leaves, flower, and buds of *Tagetes patula* L. transplants. Parađiković et al. (2017) reported that begonia transplants (*Begonia semperflorens* Link et Otto) treated with biostimulant Radifarm® had significantly higher values of all morphological parameters that were investigated (plant height, number of leaves and flowers). Biostimulant Radifarm® application improved plant growth parameters and quantitative properties of many horticultural plants such as *Capsicum annum* L.; *Ocimum basillicum* L.; *Primula acaulis* L. (Tkalec et al., 2010; Zeljković et al., 2011; Zeljković et al., 2014).

Biostimulants can be produced from various types of raw materials, such as plants and seaweed extracts, humic and fulvic substances, composts, and manures, as well as different agro-industrial wastes (Teklić et al., 2021). Biostimulant application is not studied enough, so Teklić et al. (2021) in their review tried to give an overview of recent advances in the research related to the interplay among abiotic stress, plant response, biostimulant effects

Table 1. Composition of biostimulant Radifarm® (details according to the manufacturer)

	Radifarm®				
Conc.	Organic matter (humic acids) (%)	Polysaccharides (%)	Peptides and amino acids (%)	Vitamin complex (%)	Chelated zinc (%)
w/w	30.0	7.0	12.2	0.04	0.20
w/v	37.3	8.7	14.8	0.05	0.25

and plant-derived functional food, focusing on plant metabolites as the link which connects the environment with the food chain.

In the research of González-González et al. (2020) the combination of plant biostimulant arbuscular mycorrhizal fungi and seaweed extracts, in tomato production, resulted in an additive effect and was reflected in an increase in foliar and root growth as well as in protein and carbohydrate content. Plant biostimulants which contain substances and compounds such as those derived from arbuscular mycorrhizal fungi or seaweed extracts represent an environmentally friendly tool to increase crop yield and productivity (González-González et al., 2020).

Monitored morphological parameters, plant height, plant diameter, number of leaves and flowers were, in our experiment, influenced by the year of investigation, showing that climatic conditions in 2014 were slightly more favourable for flower development which is in the horticultural production the main decorative element. Treated plants compared with control variants showed greater development of plant height and plant diameter in 2015. This can be related to the average monthly temperature in 2015. As seen in Figure 1, in the period of July-September the average monthly temperatures were slightly higher compared to 2014. This is the period which reflected by intensive plant growth, high temperature, and warm air which can additionally result in stronger plant growth.

Plants treated with biostimulant Radifarm® achieved greater fresh and dry mass of their roots and above-ground parts compared to control plants, also the year of investigation influenced the growth of transplants. The greatest fresh and dry mass of roots and above-ground parts were recorded in 2015 (Table 3).

The results of our study are similar to results of many other researchers as stated by Špoljarević et al. (2010) in their study, where biostimulant Viva® and Megafol® enhanced fresh and dry mass of *Fragaria* x *ananassa* Duch. In the research of Zeljković et al. (2010) treatment with biostimulant Radifarm® improved growth and development of roots and above ground mass for *Salvia splendens* L. transplants. Biostimulant Radifarm® was applied in the multiplication of tissue culture of *Rosa cannina* L. which increased shoot number, improved growth, and development of transplants (Tkalec et al., 2012). In the research of Ferrante et al. (2013) application of biostimulant Actiwave® increased the fresh and dry mass of *Camellia japonica* L. cuttings. The application of biostimulant based on seaweed extract, Bio-algeen S-90, has shown positive effect on growth and yield parameters after transplanting lettuce resulting in enhanced content of vitamin C (Dudaš et al., 2016).

Biostimulant solutions can be applied in different plant growth stages, from the germination phase to the phase of full plant and fruit/flower commercial maturity, by means of seed treatment, foliar application, or irrigation throughout the vegetation, as well as a

Table 2. Average values of plant height, plant diameter, number of leaves and flowers of pansy seedlings (*Viola tricolor* var. *hortensis* DC.) after treatment with biostimulant in 2014 and 2015 (means marked with different letters ^{a,b,c} significantly differ at P<0.05; ± standard error)

		Growth parameters			
		Plant height (cm)	Plant diameter (cm)	Number of leaves	Number of flowers
Biostimulant effect (A)	Treatment (A1)	6.75±0.22^a	9.27±0.20^a	20.14±0.78^a	0.60±0.07^a
	Control (A2)	6.13±0.14^a	8.56±0.11^b	17.82±0.71^b	0.49±0.11^b
Effect of year (B)	2014 (B1)	5.07±0.22^b	8.63±0.17^b	20.13±1.05^a	0.74±0.12^a
	2015 (B2)	7.81±0.14^a	9.2±0.15^a	17.83±0.13^b	0.35±0.05^b

Table 3. Average root and above-ground part fresh (FW) and dry weight (DW) of pansy seedlings (*Viola tricolor* var. *hortensis* DC.) after treatment with biostimulant in 2014 and 2015 (means marked with different letters [a,b,c] significantly differ at P<0.05; ± standard error)

		Growth parameters			
		Root FW (g)	Root DW (g)	Above ground part FW (g)	Above ground part DW (g)
Biostimulant effect (A)	Treatment (A1)	5.14±0.23[a]	0.54±0.02[a]	7.55±0.36[a]	1.07±0.03[a]
	Control (A2)	3.41±0.33[b]	0.38±0.01[b]	5.40±0.14[b]	0.75±0.03[b]
Effect of year (B)	2014 (B1)	3.72±0.26[a]	0.36±0.02[b]	6.03±0.15[a]	0.75±0.03[b]
	2015 (B2)	4.83±0.31[a]	0.56±0.01[a]	6.92±0.35[a]	1.07±0.03[a]

supplement to media in tissue culture (Parađiković et al., 2019). Carillo et al. (2020) reported positive effects of biostimulant application on plum tomatoes, in terms of enhanced contents of lycopene, asparagine and γ-aminobutyric acid. Parađiković et al. (2011) tested four commercial biostimulants in sweet yellow pepper plants, grown in a greenhouse. The results showed that natural biostimulants had a positive effect on vitamin C, total phenolic contents, improved the antioxidant activity, vitamin C, and phenolic contents in fruits. Colla et al. (2015) reported that biostimulants, when applied to the plant rhizosphere, enhanced nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and crop quality. Most reports associate better root development with the effect of humic acids on plants, probably due to its high number of oxygenated functional groups that allow better nutrient uptake (Calvo et al., 2014). Biostimulants applied in this investigation, did not contain the required nutrient concentration, but they contained macro- and micronutrients in certain amounts in which they cannot be considered as bio-fertilizers.

CONCLUSIONS

The application of biostimulant Radifarm® in the pansy (*Viola tricolor* var. *hortensis* DC.) seedling production resulted in higher growth parameters as well as significantly greater fresh and dry mass of root and above-ground parts in comparison to control plants. Under the influence of biostimulant treatment root and above ground fresh mass were 51% and 40% greater, respectively. Plants treated with biostimulant develop more roots, which is a basic postulate for better plant adaptation after transplanting. Biostimulant application also increased the most important decorative parameters for ornamental species, i.e. number and development of leaves and flowers. The number of leaves was 13% higher, and the number of flowers was 23% higher under the influence of the biostimulant treatment. Given all the above, it can be concluded that biostimulant application in pansy seedlings production can reduce fertilizer use and thereby contribute to environment preservation. Also, biostimulant application can be considered good practice by ensuring seedlings with high quality, improved plant growth and development after transplanting as well as overcoming temperature stress and providing good nutrient uptake.

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