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The Effect of Potassium Biofertilizer and Chemical Fertilizer on Quantitative and Qualitative Traits of Periwinkle (*Catharanthus roseus* Cv. 'Acillata')

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Abstract

The effect of potash biofertilizer and chemical fertilizer was studied on quantitative and qualitative traits of periwinkle in a factorial experiment based on a Randomized Complete Design with three replications, 48 experimental units, and two factors: (1) different methods of potash bio-fertilizer application at four levels of control (B_0), seed inoculation (B_1), root inoculation (B_2) and seed inoculation × root inoculation (B_3), and (2) different rates of potassium on fertigation at four levels of 0 (K_0), 100 (K_1), 200 (K_2) and 300 mg L^{-1} (K_3). It was found that the longest shelf life of 35.58 days was related to the treatment of root inoculation × 100 mg L^{-1} K. The highest number of auxiliary branches (3.66), number of leaves (42.88), plant fresh weight (17.69 g), number of flowers (4.46), and chlorophyll a (10.61 mg g^{-1} F.W.) were obtained from the treatment of seed inoculation × 300 mg L^{-1} K. The treatment of root inoculation × 200 mg L^{-1} K was the best treatment for plant potassium content (28.9 mg kg^{-1}). The highest petal anthocyanin content amounted 178.04 mg 100 g^{-1} D.W. was observed in the treatment of 'seed inoculation × root inoculation' × 0 mg l^{-1} K. The best treatment of enhancing of qualitative and quantiative traits of periwinkle was seed inoculation × 300 mg L^{-1} K.

Keywords: Biofertilizer, Petal anthocyanin, Potassium, Potassium dissolving bacteria, Shelf life.

INTRODUCTION

Soil potential for potassium supply to plants during the growing season depends on dissolved potassium on the one hand and potassium release from insoluble form to dissolved forms. So obviously, absorbable potassium does not suffice for plant growth and development and the mentioned factors should be considered (Brown *et al.,* 1993). Since the soils in Iran contain clay minerals of mica and illite, they are expected to release enough potassium to meet the plants' requirements with no need for the application of potash fertilizers. But, researchs showed that soluble K in some soils reducied fast due to plant density, K diminish and limited K fertilization. So, it is necessary to revise the use of potash fertilizers in Iranian farms (Palizdar *et al.,* 2013).

Given the importance of biological agriculture, the recognition and use of potassium-dissolving microorganisms have been considered for increasing soil available potassium. As is known, microorganisms play a vital role in transforming unavailable nutrients into nutrients available to plants so that they increase soil dissolved nutrients and crop production and quality (Keshavarz *et al.,* 2013). An example of such microorganisms is the bacteria that can transform soil mineral K into dissolved K to make it available to plants (Parma and Sindhu, 2013). There is a large population of K-releasing bacteria in soil and rhizosphere that can turn K content of soil minerals into dissolved K (Luo *et al.*, 2011; Diep and Hieu, 2013).

The studies on K dissolving microorganisms and bacteria have yielded the production of potash biofertilizers like Pota BARVAR-2 biofertilizer that contains K-releasing bacteria that releases the ions and improves K uptake by dissolving insoluble compounds around root. Many studies report higher availability or the efficiency of potassium fertilizers after applying potassium dissolving bacteria. Dorjdor *et al.* (2014) suggest that potassium dissolving bacteria can dissolve K-containing mineral silicates and release absorbable potassium for plants. Some researchers have reported that microorganisms play an important role in K cycle in nature. It is suggested that soil and rhizosphere are residing with a large population of potassium releasing bacteria that turn K content of soil minerals into soluble and available form with acids they produce (Luo *et al.*, 2011; Diep and Hieu, 2013). The effect of silicate dissolving bacteria was studied on potassium release in canola rhizosphere and it was found that the highest K uptake was devoted to plants fertilized with perfect nutrients containing silicate-dissolving bacteria. Also, it was found that the K concentration was not significantly different between plants fertilized with bacteria-free perfect nutrients and those fertilized with potassium-free, bacteria-containing nutrient, implying the significant impact of silicate dissolving bacteria on K supply to plants (Rahimzadeh *et al.*, 2013). Chen and Chen (1960) showed that the inoculation of silicate dissolving bacteria as well as potassium-containing minerals increased potassium concentration in the root zone by 25-87%. Osman (2009) reported high soil potassium solubility by the use of potassium dissolving bacteria.

In a study on safflower, Palizdar *et al.* (2013) obtained the highest plant height, stem diameter, head diameter, and branch number from the application of 150 kg ha^{-1} chemical potassium. Saber Hamishegi *et al*. (2013) reported the increase in stem length of stevia with the application of 30-40 kg K ha^{-1}. A study on the effect of nitrogen and potash fertilizers on growth, yield and alkaloidal content of periwinkle showed that the application of nitrogenous and potash fertilizers increased periwinkle's vegetative traits significantly and that the best results were obtained from the treatment of 75 kg ha^{-1} (Hassan *et al.*, 2009).

Given the importance of potassium to plants – e.g. its effect on the integrity of the stem, plant resistance to pests and diseases, and its effect on the quality of crops – the present study was carried out on potash biofertilizers (Pota BARVAR) in order to improve the vegetative and reproductive indices of periwinkle and to recommend the best treatment.

MATERIALS AND METHODS

This experiment was carried out on the basis of a Randomized Complete Design with three replications and sixteen treatments. Each plot was composed of five pots and each pot contained one plant. The studied treatments included different application methods of Pota BARVAR-2 biofertilizer [no biofertilizers (B_0), inoculation of seeds (B_1), inoculation of roots (B_2) and inoculation of seeds × inoculation of roots (B_3)] and four levels of pure chemical potassium on fertigation (from Crystalon source with 20-20-20 ratio of NPK) [0 (K_0), 100 (K_1), 200 (K_3), and 300 (K_4) mg L^{-1}].The seeds of periwinkle (*Catharanthus roseus* cv. 'Acillata') were used with 90% viability. They were first treated according to the plan, and then were sown in the medium containing garden soil mixed with leaf mold and river sand. Table 1 presents the characteristics of the medium.

To inoculate the seeds, a 100-g package of biofertilizers was solved in two liters of water. It was filtered 10 minutes later, and the filtered solution was diluted with five liters of water. Then, the seeds intended for inoculation were placed in the (filtered) fertilizer for five minutes (Fig. 1a) and then, they were sown in pots (Hashemabadi *et al.*, 2012). Forty days later, the seedlings were transferred to main pots with a mouth diameter of 14 cm containing garden soil mixed with leaf mold and river sand (Table 1). To inoculate the roots, the procedure for the preparation of fertilizer solution was repeated. Then, the plants planned for root inoculation were taken out of the seedling pots and were placed in the filtered fertilizer for five minutes (Fig. 1b) (Hashemabadi *et al.*, 2012). Then, the treated plants were transferred to main pots and were placed in open air at 25-30°C until the end of the experiment. The plants were irrigated every two day and were fertilized with Crystalon 20-20-20 once 15 days until the end of the experiment (i.e. for five months).

The recorded traits included plant and medium potassium content, shelf life, the number of flowers per plant, the number of auxiliary branches, the number of leaves per plant, plant fresh and dry weight, leaf chlorophyll, and petal anthocyanins. Potassium was measured by flame photometry method (Worth, 1985). The days from the emergence of the first bud until 50% wilting of the flowers on the plants were counted as shelf life. The number of flowers per plant, auxiliary branches and leaves per plant were measured once five days until the end of the shelf life(50% wilting of flowers), and then, the readings were averaged. At the end of the experiment (the end of shelf life), the plants were removed from the soil, and their fresh weight was weighed with a 0.01 g-precision digital scale. Then, they were oven-dried at 70°C for 24 hours to measure their dry weight. To estimate chlorophyll, the leaves were cut at the end of shelf lifeand, their chlorophyll was measured by Mazumdar and Majumdar (2003)'s method. Finally, leaf chlorophyll a, b and total was calculated by the following equation:

Table 1. The physical and chemical characteristics of medium used in the present study.

Soil texture	Organic carbon (%)	N (%)	Available P (mg kg^{-1})	Available K (mg kg^{-1})	pH
Sandy loam	13.45	9.4	20.5	34.2	7.16

Fig. 1. Seed and root treatment with PotaBARVAR biofertilizer: (a) seed inoculation, and (b) root inoculation.

$$\text{Chlorophyll a (mg g F.W.)} = 9.93(A_{660}) - 0.777(A_{642})$$

$$\text{Chlorophyll b (mg g F.W.)} = 17.6(A_{642}) - 2.81(A_{660})$$

$$\text{Total chlorophyll (mg g F.W.)} = 7.12(A_{660}) - 16.8(A_{642})$$

Petal anthocyanin was estimated by spectrophotometry and the following formula:

$$\text{Petal anthocyanin (mg 100 g F.W.)} = \frac{e \times b \times c}{d \times a} \times 100$$

Where, a, b, c, d, and e were the reading, sample size, whole solution, taken sample size, and sample weight, respectively.

Data were analyzed with MSTAT-C Statistical Software Package, the means were compared by LSD method, and the graphs were drawn with MS-Excel Software Package.

RESULTS

The analysis of variance showed that the effect of interactions of different potash biofertilizer application methods and different chemical fertilizer rates was significant at 1 probability level on the plant k, shoot fresh weight, leaf number, petal anthocyanins and chlorophyll a of periwinkle. Interactions of different potash biofertilizer application methods and different chemical fertilizer rates was significant at 5 probability level on the shelf life, flower number and shoot dry weight of periwinkle (Table 2).

Potassium concentration (medium)

Results showed that interaction between application methods of biofertilizer and different concentration of K^+ wasnot difference significantly, Table 2 shows that all interactions between the treatments resulted in higher medium potassium than control, so that B_2K_2 and B_1K_3 produced the highest medium potassium of 101.1 mg kg^{-1}. The lowest one (41.1 mg kg^{-1}) was related to the treatment of B_0K_0 (Table 3).

Potassium concentration (plant)

According to means comparison for the interactions between different methods of potash biofer-

Table 2. Analysis of variance (ANOVA) of the effect of different application methods of bio-fertilizer and different levels of chemical potash on plant K, medium K, shelf life, flower number and shoot dry weight, shoot fresh weight, leaf number, petal anthocyanins and chlorophyll a, b and totalof periwinkle.

S.o.V	df	Plant K (mg kg^{-1})	Media K (mg kg^{-1})	Shelf life (day)	Flower number	Shoot fresh weight (g)	Shoot dry weight (g)	Leaf number	Branch number	Petal anthocyanins (mg 100g $^{-1}$DW)	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Total chlorophyll (mg g^{-1} FW)
B	3	8.817*	15.43**	16.67*	1.929*	15.13**	1.193**	143.76**	3.00*	3061.3**	2.16**	2.198**	6.99**
K	3	19.10**	19.78**	32.44**	0.769ns	36.66**	1.107**	141.53**	1.95**	3139.9**	49.78**	12.60**	109.2**
B*K	9	29.23**	0.557ns	10.46*	0.620*	9.41**	0.398*	46.80**	1.17**	2327.6**	2.00**	0.882ns	1.174ns
Error	32	2.19	0.624	5.32	0.284	2.33	0.160	15.4	0.33	28.33	0.351	0.477	0.839
CV (%)		6.02	12.26	7.21	17.99	13.76	15.44	14.04	30.5	4.08	7.60	14.21	7.23

**: Significant at = 1%, *: Significant at = 5%, ns= Not significant.

Table 3. Means comparison for the simple effects and interactions of potash biofertilizer and potassium chemical fertilizer for the recorded traits.

Treatments	Plant K (mg kg-1)	Medium K (mg kg-1)	Shelf life (day)	Flower number	Shoot fresh weight (g)	Shoot dry weight (g)	Leaf number	Branch number	Petal anthocyanins (mg 100g -1DW)	Chlorophyll a (mg g-1 FW)	Chlorophyll b (mg g-1 FW)	Total chlorophyll (mg g-1 FW)
B_0	23.68^{c}	48.60^{c}	30.33^{b}	2.60^{b}	10.17^{b}	2.25^{c}	23.77^{c}	1.35^{c}	114.60^{d}	7.35^{c}	4.31^{b}	11.67^{b}
B_1	24.05^{bc}	70.10^{ab}	33.05^{a}	3.53^{a}	12.74^{a}	3.01^{a}	32.24^{a}	2.55^{a}	119.56^{c}	7.87^{ab}	5.35^{a}	13.23^{a}
B_2	25.27^{ab}	64.20^{b}	32.52^{a}	2.81^{b}	10.73^{b}	2.49^{bc}	27.99^{b}	1.71^{bc}	148.36^{a}	8.34^{a}	4.94^{a}	13.28 ^{a}
B_3	25.38^{a}	74.60^{a}	31.93^{ab}	2.90^{b}	10.80^{b}	2.61^{b}	27.74^{b}	1.91^{b}	139.10^{b}	7.58^{bc}	4.83^{ab}	12.42^{b}
K_0	22.92^{c}	50.30^{d}	29.96^{c}	2.80^{a}	9.32^{c}	2.24^{b}	26.13^{bc}	1.49^{b}	134.50^{b}	5.43^{d}	3.40^{c}	8.84 ^{d}
K_1	25.71^{a}	58.50^{c}	33.98^{c}	2.70^{a}	10.26^{bc}	2.43^{b}	24.46^{c}	1.58^{b}	151.06^{a}	6.90^{c}	4.92^{b}	11.82 ^{c}
K_2	25.42^{ab}	68.80^{b}	32.98^{ab}	3.11^{a}	11.47^{b}	2.80^{a}	28.83^{b}	2.19^{a}	122.22^{c}	8.76^{b}	5.37^{ab}	14.14 ^{b}
K_3	24.33^{b}	80.00^{a}	31.79^{bc}	3.23^{a}	13.37^{a}	2.88^{a}	32.33^{a}	2.27^{a}	113.84^{d}	10.06^{a}	5.73^{a}	15.79 ^{a}
B_0K_0	19.91^{i}	41.10^{a}	29.66^{e}	3.26^{bc}	7.70^{f}	1.94^{f}	25.10^{def}	0.99^{fg}	76.64^{h}	4.42^{h}	2.48^{a}	7.40^{a}
B_0K_1	24.75^{def}	51.10^{a}	29.24^{e}	2.53^{cde}	8.25^{ef}	2.14^{def}	19.55^{f}	0.88^{g}	137.95^{c}	7.65^{d}	4.05^{a}	11.51^{a}
B_0K_2	26.43^{a-d}	66.70^{a}	29.56^{e}	2.16^{ef}	11.64^{b}	2.31^{c-f}	21.55^{ef}	1.44^{d-g}	140.65^{c}	8.71^{bc}	4.66^{a}	13.52^{a}
B_0K_3	27.70^{abc}	71.40^{a}	29.33^{e}	2.73^{b-e}	11.75^{b}	2.14^{def}	28.88^{bcd}	2.10^{b-e}	86.17^{g}	8.63^{bc}	5.73^{a}	15.02^{a}
B_1K_0	22.64^{fgh}	51.90^{a}	35.36^{ab}	3.40^{b}	10.25^{b-e}	2.06^{ef}	29.44^{bcd}	1.88^{c-f}	124.40^{d}	5.39^{g}	3.75^{a}	9.47^{a}
B_1K_1	25.61^{cde}	78.90^{a}	34.63^{abc}	2.86^{bcd}	11.98^{b}	2.39^{c-f}	27.77^{b-e}	2.10^{b-e}	138.50^{c}	6.04^{fg}	4.27^{a}	10.94^{a}
B_1K_2	24.39^{d-g}	99.90^{a}	34.13^{abc}	4.03^{b}	11.04^{bcd}	2.69^{b-e}	28.88^{bcd}	2.55^{bc}	93.63^{g}	9.47^{b}	5.63^{a}	15.40^{a}
B_1K_3	26.29^{bcd}	101.10^{a}	34.20^{abc}	4.46^{a}	17.69^{a}	3.42^{a}	42.88^{a}	3.66^{a}	138.71^{c}	10.61^{a}	5.77^{a}	16.66^{a}
B_2K_0	21.05^{hi}	63.50^{a}	31.40^{cde}	2.46^{ef}	8.57^{def}	2.24^{c-f}	26.66^{cde}	0.99^{fg}	141.96^{c}	6.72^{ef}	3.68^{a}	10.53^{a}
B_2K_1	26.39^{bcd}	73.40^{a}	35.58^{a}	3.06^{bcd}	11.80^{b}	2.70^{b-e}	24.44^{def}	1.77^{c-g}	167.35^{b}	7.26^{de}	4.82^{a}	12.20^{a}
B_2K_2	28.90^{a}	101.10^{a}	31.80^{b-e}	3.06^{bcd}	11.07^{bcd}	2.89^{abc}	29.10^{bcd}	1.77^{c-g}	123.11^{de}	8.93^{b}	5.49^{a}	14.59^{a}
B_2K_3	24.76^{def}	71.60^{a}	31.33^{cde}	2.66^{b-e}	11.48^{bc}	2.51^{c-f}	31.77^{bc}	2.33^{bcd}	161.02^{b}	10.45^{a}	5.76^{a}	16.31^{a}
B_3K_0	20.65^{hi}	89.00^{a}	33.36^{a-d}	3.06^{bcd}	10.77^{b-e}	2.73^{bcd}	25.55^{c-f}	1.55^{d-g}	178.04^{a}	5.21^{gh}	3.71^{a}	8.97^{a}
B_3K_1	28.48^{ab}	98.90^{a}	32.31^{a-e}	2.33^{de}	9.02^{c-f}	2.51^{c-f}	23.88^{def}	1.22^{efg}	160.45^{b}	6.64^{ef}	4.56^{a}	11.87^{a}
B_3K_2	21.95^{ghi}	87.00^{a}	29.10^{e}	2.93^{bcd}	12.15^{b}	3.33^{ab}	28.44^{bcd}	1.88^{c-f}	114.95^{e}	7.93^{cd}	5.06^{a}	13.97^{a}
B_3K_3	23.66^{efg}	91.80^{a}	30.35^{de}	3.06^{bcd}	12.58^{b}	3.47^{a}	33.11^{b}	2.99^{ab}	102.97^{f}	10.55^{a}	5.33^{a}	16.65^{a}

* In each column, means with the similar letter(s) are not significantly different at 5% level of probability using LSD test. B0: Without bio-fertilizer, B1: Inoculation of seed, B2: Inoculation of root, B3: Inoculation of seed and root with bio-fertilizer. K_0-K_3: Different levels of potassium; K_0: 0 mg l^{-1}, K1: 100 mg l^{-1}, K_2: 200 mg l^{-1}, and K_3: 300 mg l^{-1}.

tilization and different rates of chemical potassium, the treatment of B_2K_2 produced the highest plant potassium content of 28.90 mg kg^{-1} which had no significant difference with the treatments of B_3K_1 (28.48 mg kg^{-1}), B_0K_3 (27.70 mg kg^{-1}), and B_0K_2 (26.43 mg kg^{-1}). The lowest plant potassium content of 19.91 mg kg^{-1} was seen in the treatment of B0K0 (Table 3).

Shelf life

Table 2 reveals that among the treatments, the highest shelf life was devoted to the treatments of B_2K_1 (35.58 days), B_1K_0 (35.36 days), B_1K_1 (34.63 days), B_1K_2 (34.13 days), B_1K_3 (34.20 days), B_3K_1 (32.31 days), and B_3K_0 (33.36 days) with no statistically significant differences to each other. The lowest shelf life was resulted from the treatments of B_0K_0 (29.66 days), B_0K_3 (29.33 days), B_0K_1 (29.24 days), B_0K_2 (29.56 days), or B_3K_2 (29.10 days) with no statistically significant differences (Table 3).

* In each column, means with the similar letter(s) are not significantly different at 5% level of probability using LSD test. B_0: Without bio-fertilizer, B_1: Inoculation of seed, B_2: Inoculation of root, B_3: Inoculation of seed and root with bio-fertilizer. K_0K_3: Different levels of potassium; K0: 0 mg l^{-1}, K_1: 100 mg l^{-1}, K_2: 200 mg l^{-1}, and K3: 300 mg l^{-1}.

Number of branches

Means comparison for the interactions between different methods of potash biofertilization and different rates of chemical potassium revealed that the highest number of branches was obtained from interactive treatments of all four methods of potash biofertilizer application × 300 mg L^{-1} K. As is evident in Table 2, the highest number of branches (3.66) was related to the treatment of B_1K_3, which had no statistically significant differences with the treatment of B_3K_3 (2.99 branches). The lowest number of branches (0.88) was obtained from the treatment of B_0K_1 (Table 3).

Number of leaves

As means comparison of the interactions for periwinkle leaf number showed, the application of chemical potassium along with all four methods of biofertilizer application resulted in the loss of leaf number, but as K fertilization rate was increased, leaf number was improved. As shown in Table 2, the highest number of leaves (42.88) was related to the treatment of B_1K_3 and the lowest one (19.55) to the treatment of B0K1 (Table 3).

Number of flowers

The means comparison results for the interactions between different methods of potash biofertilizer application and different rates of chemical potassium indicated that the highest number of flowers (4.46 flowers) was produced under the treatment of B_1K_3. The lowest ones were obtained from the treatments of B_1K_2 (2.16) and B_2K_0 (2.46) with no statistically significant differences (Table 3).

Plant fresh weight

It was found that the highest plant fresh weight (17.69 g) was related to B_1K_3 and the lowest one to the treatments of B_0K_0 (7.70 g) and B_0K_1 (8.25 g) (Table 3).

Plant dry weight

The best treatments for plant dry weight were B_1K_3 (3.42 g) and B_3K_3 (3.47 g) with no significant difference with the treatments of B_3K_2 (3.33 g) and B_2K_2 (2.89 g). The lowest plant dry weight was produced under the treatments of B_0K_0 (1.94 g), B_0K_1 (2.14 g) and B_0K_3 (2.14 g) (Table 3).

Chlorophyll a

According to the results, chlorophyll a was increased with chemical K rate under all four methods of biofertilizer application. Among all treatments, B_0K_0 produced the lowest chlorophyll a of 4.42 mg g^{-1} F.W.. The highest one was obtained from the treatments of B_1K_3 (10.61 mg g^{-1} F.W.), B3K3 (10.55 mg g^{-1} F.W.), and B_2K_3 (10.45 mg g^{-1} F.W.) with no significant differences to each other (Table 3). The maximum content of chlorophyll b was obtained for B_1K_3 (16.66 mg g^{-1} F.W.) and B_3K_3 (16.65 mg g^{-1} F.W.). The maximum content of total chlorophyll was also related to treatment of B_1K_3 (5.77 mg g^{-1} F.W.) and b_2k_3 (5.76 mg g^{-1} F.W.)(Table 2).

Petal anthocyanin

As means comparison showed, the highest petal anthocyanin of 178.04 mg 100 g^{-1} D.W. was devoted to the treatment of B_3K_0 and the lowest one to the treatment of B_0K_0 (76.64 mg 100 g^{-1} D.W.) and then, to the treatments of B_0K_3 (86.17 mg 100 g^{-1} D.W.) and B_1K_2 (93.63 mg 100 g^{-1} D.W.) with no significant differences with each other (Table 3).

DISCUSSION

As mentioned before, the application of potash biofertilizers, resulted in significantly higher potassium content in medium and plant than in control. In fact, it can be said that the activity of potassium dissolving bacteria in soil increased soil dissolved potassium and its higher uptake rate by plants. In a study on the capability of potassium dissolving bacterial in soil, Osman (2009) reported higher rate of K release by of the bacteria. Dorjdor *et al*. (2014) suggested that potassium dissolving bacteria (Bacillus, Paenibacillus and Pseudomonas) improved available K and plant growth and efficiency by increasing absorbable potassium solubility and release. In a factorial study on the effect of silicate dissolving bacteria on potassium release in canola farm with two factors including nutrient solution (complete or K-free nutrient solution) and bacteria (with and without bacteria), Rahimzadeh *et al.* (2013) observed that potassium dissolving bacteria significantly affected potassium supply to plants. Also, under K-free nutritional conditions, plants treated with potassium dissolving bacteria absorbed more potassium than those lacking bacteria.

Researchers believe that higher potassium application boosts potassium concentration in soil solution increasing potassium dissipation on root surface. Then, plants uptake more potassium, and its concentration is increased in their tissues (Malakouti and Homaee, 2004; Malakooti *et al.*, 2008).

As mentioned above, most estimated traits (shelf life, flower number, auxiliary branch number, leaf number, and plant fresh and dry weight) were increased by the application of potash chemical fertilizer and the biofertilizers. Higher vegetative traits with potash the biofertilizers application can be related to the production of growth stimulators by the activity of potassium dissolving silicate bacteria. It is revealed that there are a lot of gibberellins and other activators which are plant growth stimulators in potassium dissolving bacteria culture. Researchers suggest that the favorable impact of potassium on plant growth is related to the role of potassium as an active osmotic element affecting water uptake and also, its intervention in cell division and the conversion of big cells into small cells and its effects on growth components. On the other hand, the oligodynamic role of potassium (activator of kinases) and its intervention in photosynthesis should not be ignored (Ebrahimzadeh, 1978; Malakouti, 2000).

Niakan *et al*. (2004) stated that potassium and phosphorous improved the effect of nitrogen on plants by their oligodynamic impacts (participation in energy transfer processes, activation of kinases, and contribution to water osmotic absorption) resulting in better photosynthesis and vegetative traits of peppermint. Some researchers argue that in addition to increasing nutrients uptake in plants, biofertilizers improve plant growth and yield through the biosynthesis of plant hormones, control of plant pathogens, and some other mechanisms (Afzal *et al.*, 2005; Khalid *et al.*, 2004; Mehana and Vahid, 2002).

Hassan *et al.* (2009) studied the effect of nitrogen and potassium fertilization on growth, yield and alkaloidal content of periwinkle. They used N fertilizer at three rates of 50, 100 and 150 kg ha^{-1} and K fertilizer at three rates of 25, 50 and 75 kg ha^{-1}. They reported that nitrogen and potash fertilization increased plant height, number of branches per plant, plant dry and fresh weight, and herb yield significantly as compared to control. These traits were increased more at higher potash fertilization rates, so that all traits except plant height had their highest records at a K rate of 75 kg ha^{-1}.

Karimi *et al.* (2009) reported that plants fertilized with 1 mM potassium had the highest growth and that fertilization with higher rates of potassium reduced the growth of sesbania acleate plants, so that the highest fresh weight (191.70 g), root dry weight (3.46 g), stem dry weight (5.93 g), leaf dry weight (8.02 g), and total dry weight (17.42 g) were obtained from the treatment of 1 mM potassium and the lowest ones were obtained from the treatment of 40 mM potassium. Also, as potassium rate was increased, the potassium contents of stem, leaf and root were increased.

It is shown that the number of auxiliary branches of oil flax was reduced with the increase in potassium level, though the reduction was not statistically significant (Parhizkar-Khajani *et al.*, 2012). Csizinsky (1999) reported that the highest fresh weights of parsley and oregano were produced under the application of 40-80 kg ha^{-1} of potash fertilizer. Azizabadi *et al*. (2014) reported that higher potassium rates resulted in higher shoot dry weight and that the highest shoot dry weight of 5.43 g was associated with the application of 230 mg K kg^{-1}. In a study on pepper by Fawazy *et al*. (2007) and on tomato by Nanadal *et al*. (1998), it was shown that potassium influenced plant dry weight significantly.

The application of potassium biofertilizer and chemical fertilizer helped conserving and increasing leaf and petal pigments of periwinkle in the present study. Azizabadi *et al*. (2014) reported that potassium application increased chlorophyll index and that the highest chlorophyll (68.77%) was obtained from the treatment of 230 mg K kg^{-1}, which is in agreement with our findings. Kumar and Kumar (2008) found that higher potassium rates resulted in higher leaf chlorophyll. They suggested that higher photosynthetic activities resulted from higher relative chlorophyll content of leaves can be related to the role of potassium in the synthesis of chlorophyll pigments precursor. Marius *et al*. (2005) reported that microbial inoculation increased pigments of sunflowers. Han and Lee (2005) found that lettuce inoculation with growth promoting bacteria increased growth and chlorophyll content of leaves, which is consistent with our findings.

CONCLUSION

Given the importance of periwinkle as an ornamental-medicinal plant, it is crucial to meet its nutrient requirements by non-chemical and biological methods. In the present study, potash biofertilizer improved periwinkle growth by increasing the solubility and uptake of nutrients the plant needs, so that among interactions of potash biofertilizer application method × different chemical fertilizer rates, the inoculation of roots × 100 mg L^{-1} chemical potassium was found to be the best treatment for shelf life as an important index in the cultivation of ornamental flowers although it had no significant difference with seed inoculation treatment at all four levels of 0, 100, 200 and 300 mg L^{-1} chemical potassium. Overall, these treatments were amongst the best treatments for the studied traits. In summary, it can be concluded that the potash biofertilizers application as seed and root inoculation at low chemical fertilization rates is appropriate for periwinkle growth. However, further studies are recommended in this field.

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