Growth response of *Viola odorata* L. induced by arbuscular mycorrhizal fungi and *Pseudomonas fluorescens*

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Abstract
This investigation was carried out in a poly-house, Department of Botany, Kurukshetra University, Kurukshetra, India, to verify the efficacy of dominant arbuscular mycorrhizal fungi (Glomus mosseae and Acaulospora laevis) and phosphate solubilizing bacteria (Pseudomonas fluorescens) as bio-fertilizers either independently or in combination on uptake of nutrient, vegetative growth as well as flower yield of Viola odorata L. grown in earthen pot. Our findings suggest that all co-inoculation treatments showed beneficial effects on all the growth, nutrient uptake, phosphatase activity and flower yield of V. odorata.

The results of present investigation also demonstrate that co-inoculation of biofertilizers like Pseudomonas fluorescens with AM fungi, significantly promotes higher root colonization and spore density enhancing the phosphorus (P) uptake and improving the content of photosynthetic pigments.

Keywords: Arbuscular mycorrhizal fungi, biomass, flower yield and *Viola odorata*.

Introduction

Viola odorata is an evergreen perennial herb species native to Asia and Europe, but has also been imported to Australia and North America. It is commonly known as Sweet Violet and Banaksa in India. *V. odorata* is widely grown as ornamental and medicinal plant, used as remedy to cure sore throat and tonsillitis. The leaves and flowers of *V. odorata* are made into syrup used for respiratory ailments associated with congestion, coughing and sore throat[1]. Below ground part contains an alkaloid called violine which is used as emetic (causing vomiting) and expectorant agent[2,3]. *V. odorata* provides best antimicrobial activity against *Proteus vulgaris* and *Escherchia coli*[4]. The herbal tea made from the whole plant parts is utilized to cure digestive ailments whereas new research has detected the presence of natural aspirin (glycoside of salicylic acid) which substantiates its use for centuries as a medicinal remedy for migraine, body pains and as a sedative.

In spite of its medicinal value, its biomass production was highly reduced due to low seed germination as a result of severe seed dormancy, limited growth or over exploitation in wild condition and lack of appropriate strategy for successful establishment of the transplanted sapling from wild condition to nursery field. Inter-specific or intra-specific hybridization in natural condition is another promising factor that necessitates its preservation in controlled condition[5].

A high demand of therapeutic plants for their curative potential is not uncommon, especially those grown in wild conditions. Like other therapeutic plants, *Viola odorata* equally drew attention of growers and researchers toward its cultivation, preservation and biomass production to fulfill required demand through utilization of modern technique. To compensate increasing demand of therapeutic plants, recent research has focused on development of appropriate strategy to ensure not only the establishment of plant in controlled condition but also to improve its productivity.

During cultivation practices, inoculation of plant with native arbuscular mycorrhizal fungi and its proper management is a beneficial tool to improve establishment rate and growth by minimising the limiting effect of nutrients, to develop resistance against pathogen and to influence the concentration of secondary metabolites in plant[6,7]. During early stage of plant growth, AM symbiosis is helpful to improve plant survival rate and growth through wide range of chemical and biological reactions in the rhizosphere and plant tissues including production of plant hormones, hydrolytic enzymes to kill pathogen residing in rhizospheric soil, alteration in root exudates, affecting solubility of soil nutrients and interaction with other soil microbes[8-11].

Use of arbuscular mycorrhizal fungi is also helpful to make soil more preferable for growth of medicinal plant by improving soil particle aggregation, soil fertility and water holding capacity of the soil. Irrespective to beneficial role of AM fungi, plant growth promoting bacteria belong to another group of soil microbes having immense potential to mobilize inorganic soil phosphates through acidification of microbial cell; improve nitrogen fixation ability of plant; synthesize phytohormones like auxin, gibberiline and ethylene; solubilise potassium; produce siderophores; synthesize antibiotics and also be helpful in development of plant disease resistance[12-14].

In spite of affecting plant growth, PGPR has regulatory control over mycorrhizal spore establishment inside the host tissue as fungal hyphae, hyphal growth as well as its functioning[15]. Arbuscular mycorrhizal association and some plant growth promoting bacteria by making symbiotic

association can be helpful for obtaining appropriate amount of absorbable phosphorus from organic matters and minerals by production of phosphatase enzymes as well as increased diffusion zone around root architecture[16]. AM associations and plant growth promoting bacteria have been reported to have functions in improving the growth of medicinal plants and the productivity of medicinal compounds[17-21].

The present study was performed to make an inventory on consequent effect of Arbuscular mycorrhizal fungi i.e. *Glomus mosseae* and *Acaulospora laevis* either independently or in various combinations with *P. fluorescens* to find out the best combination having maximum potential of increasing plant growth, flower yield and P uptake in viola plant.

Material and Methods

Mass multiplication of bioinoculants: The dominant AM fungal (*Glomus mosseae and Acaulospora laevis*) spores were isolated from the rhizospheric soil of naturally grown *Viola sp*. by using wet sieving and decanting technique[22] identified by using keys[23].

The isolated dominant AM fungal spores were multiplied with wheat as host by Funnel technique given by Menge and Timmer[24] for three months. The inoculum of *Pseudomonas fluorescens* (MTCC NO. 103) was obtained from the Institute of Microbial Technology, Chandigarh, India and multiplied by using nutrient broth medium having beef extract: 3g, peptone: 5g, NaCl: 5g, 1000 mL distilled water, incubate for 48 hours at 32˚C to obtain concentration of 1×10^9 colony forming units (cfu) ml^{-1}.

Plant material: The saplings were procured from Herbal Garden Neri Hamirpur, Himachal Pradesh India. These saplings were 45 days old after germination and have four to five leaves. Before sowing, the roots of all saplings were washed with distilled water. In case of control and treatment without *P. fluorescens*, plants were directly transplanted after washing. In treatment with *P. fluorescens*, roots were placed in cell suspension of *P. fluorescens* for 2 to 3 minutes before transplanting them in to earthen pots.

Experimental site and setup: The investigation was performed in poly-house that received natural sunlight with temp (25°±5 C) and humidity (50-70%) in the herbal garden of Botany Department, Kurukshetra University, Kurukshetra, Haryana during January to April 2017. The loamy soil used in investigation consisted of sand-73.5%, silt-21.80%, clay-4.70%, pH-8.08 ±0, EC-0.24 dS/m, organic carbon-0.40%, total N- 0.042%, P- 7.31 kg/acre, K-87 kg/acre and S- 14.80ppm. The experiment was laid out in a randomized complete block design with five replicates for each treatment. Top soil (0-30 cm) was collected from an investigational site, sieved through 2mm sieve and mixed with sand in a proportion of 1:3 (sand:soil) and soil mixture sterilized in autoclaved for 2 hours at 121°C and at 15psi.

The earthen pots (15x15 cm) were selected having capacity of 2 kg soil. The saplings were transplanted in each of the earthen pots having sterilized soil and 10% w:w selected inoculums of AM fungi. Plants were grown under natural illumination and watered regularly in a poly-house. The experiment was performed with below listed treatments:

1. Control (autoclaved soil mixture without any bioinoculant)
2. *Acaulospora laevis* (A)
3. *Glomus mosseae* (G)
4. *Pseudomonas fluorescens* (P)
5. *A. laevis* + *G. mosseae* (A +G)
6. *A. laevis* + *P. fluorescens* (A + P)
7. *G. mosseae* + *P. fluorescens* (G + P)
8. *G. mosseae* + *A. laevis* + *P. fluorescens* (G+ A + P)

Each treatment had five replicates and a single plant was grown in each pot of replicate.

Harvest and analysis: After 120 days, five plants of each replicate were analyzed for different growth and physiological parameters. The root and shoot were harvested separately and fresh and dry weight was taken. Shoot and root length (cm) were measured with the help of scale. Percentage root colonization was assessed by Rapid Clearing and Staining Technique of Phillips and Hayman[25].

AM spores were isolated by Wet Sieving and Decanting Technique of Gerdemann and Nicolson[22]; shoot and root phosphorus content were determined by vanado-molybdo-phosphoric acid yellow colour method; acidic and alkaline phosphatase activity of fresh roots was determined by Tabatabai and Bremner[26]; total chlorophyll content was estimated by using Arnon's method[27].

Statistical analysis: The data was subjected for statistical analysis by using Analysis of Variance (ANOVA) followed by post hoc test performed by SPSS software package SPSS 16.0 (SPSS Inc. Chicago, IL). Duncan's multiple-range test (DMRT) was performed at $P \leq 0.05$ on each of the significant variables measured.

Results

Effects on vegetative parameters: It was found that the mycorrhizal plant had pronounced vegetative parameters with respect to the non-mycorrhizal plant after 120 days of inoculation. Maximum crown size was recorded in plants treated with *Glomus mosseae*, *Acaulospora laevis* and *Pseudomonas fluorescens* (G+A+P) (9.89±3.38 cm) and least in control (7.56±2.1cm) as shown in table 1. The shoot fresh weight was noticed highest in plants inoculated with *A. laevis* and *P. fluorescens* (4.82±0.93 g) while dry weight in *G. mosseae+A. laevis +P. fluorescens* treatment (3.99±0.45g).

Plants treated with *Glomus* show maximum increment in root architecture in terms of length; root biomass comprises fresh weight (14.0±3.55g) and dry weight (6.88±0.82 g).

Also, the leaf number and leaf area were much more in mycorrhizal plant than control plant (Table 2). It was observed that both leaf number (25.33±4.72) and leaf area (18.2±1.18 cm^2) were found maximum in *G. mosseae* + *A. laevis* + *P. fluorescens* treatment.

AMF spore density and root colonization rate: It is clearly evident from the present investigation that AM spores readily responded to host plant and not only enhance plant growth but also self proliferate under the influence of symbiotic association with host. The saplings were grown under different treatments with AM fungi and *P. fluorescens* both independently or in combination showed better mycorrhization status in their roots except plant augmented with *P. fluorescens* alone and in control. The extent of mycorrhization was evaluated by assessment of percentage of root colonization and spore density that varied with different treatments.

Table 1
Interactive effect of AM fungi and *P. fluorescens* on growth response of *V. odorata* after 120 days

Parameters Treatments	Crown size (cm)	Shoot weight(g)		Root length (cm)	Root weight(g)	
		Fresh	Dry		Fresh	Dry
Control	7.56±2.11^{a}	1.76±0.68^{b}	1.58±0.28^{b}	17.26±3.30^{e}	4.53±0.04^{e}	1.28±0.28^{d}
Glomus mosseae	9.38±3.58^{a}	3.79±1.83^{ab}	2.61±0.58^{b}	36.43±0.70^{a}	14.0±3.55^{a}	6.88±0.82^{a}
Acaulospora laevis	8.48±1.54^{a}	2.55±0.65^{ab}	2.28±0.51^{b}	34.36±0.76^{a}	10.6±1.02^{b}	2.90±0.48^{bc}
Pseudomonas fluorescens	8.98±1.40^{a}	2.87±0.52^{ab}	1.73±0.07^{b}	21.40±0.91^{cd}	6.11±2.00^{de}	4.17±1.09^{b}
A. laevis+ *G. mosseae*	8.20±1.91^{a}	2.76±1.65^{ab}	2.15±0.45^{b}	24.73±3.90^{bc}	6.51±0.46^{cde}	3.07±0.75^{bc}
A. laevis + *P. fluorescens*	7.90±1.80^{a}	4.82±0.93^{a}	2.23±0.77^{b}	22.63±0.83bc	9.15±1.06^{bc}	3.87±0.07^{bc}
G. mosseae + *P.fluorescens*	8.10±4.07^{a}	2.80±0.26^{ab}	2.06±0.73^{b}	18.6±2.86^{de}	8.09±0.18^{bcd}	2.59±0.04^{c}
G. mosseae+ *A. laevis* + *P. fluorescens*	9.89±3.38^{a}	4.03±2.84^{ab}	3.99±0.45^{a}	25.8±0.60^{b}	10.5±0.73^{b}	3.15±0.92^{bc}
L.S.D (P≤0.05)	0.6333	2.663	0.920	3.737	2.710	1.165
ANNOVA F(7,16)	0.265	1.424	5.839	31.099	11.407	17.397

G†: *G. mosseae*, A: *A. laevis*, P: *P. fluorescens*, ‡: Each value is a mean of five replicates, ±: Standard deviation, AM: Arbuscular mycorrhizae, Values in columns followed by same letter are not significantly different, P≤0.05, least significant difference test

Table 2
Interactive effect of AM fungi and *P. fluorescens* on Leaf number, Leaf area and mycorrhization in *V. odorata* after 120 days

Parameters Treatments	Leaf number	Leaf Area (cm^2)	AM spore number/10 g of soil	AM root colonization (%)
Control	10.66±1.52^{c}	3.03±0.60^{f}	0^{f}	0^{c}
Glomus mosseae	18.00±4.00^{b}	6.60±0.10^{d}	54.63±5.783^{de}	46.2±13.05^{b}
Acaulospora laevis	15.33±1.52^{bc}	4.46±1.15^{ef}	45.23±4.860^{e}	44.3±04.20^{b}
Pseudomonas fluorescens	22.00±3.00^{ab}	5.73±0.92^{de}	0^{f}	0^{c}
A. laevis+ *G. mosseae*	19.00±6.00^{ab}	8.33±0.37^{c}	66.29±7.056^{cd}	49.82±05.02^{b}
A. laevis + *P. fluorescens*	17.00±4.00^{bc}	8.96±0.66^{c}	81.86±13.32^{ab}	75.86±16.61^{a}
G. mosseae + *P. fluorescens*	21.00±2.00^{ab}	15.0±1.21^{b}	77.96±12.74^{bc}	78.26±10.68^{a}
G. mosseae+ *A. laevis* + *P. fluorescens*	25.33±4.72^{a}	18.2±1.18^{a}	92.73±5.598^{a}	85.70±14.04^{a}
L.S.D (P≤0.05)	6.359	1.504	13.365	17.998
ANNOVA F(7,16)	4.423	110.452	63.72	32.732

G†: *G. mosseae*, A: *A. laevis*, P: *P. fluorescens*, ‡: Each value is a mean of five replicates, ±: Standard deviation, AM: Arbuscular mycorrhizae, Values in columns followed by same letter are not significantly different, P≤0.05, least significant difference test

Both AM spore density and percent AM root colonization were recorded highest (92.73±5.598 and 85.70±14.04) in triple inoculation of bio-inoculants i.e. *G. mosseae* + *A. laevis* + *P. fluorescens* followed by dual inoculation of *G. mosseae* + *P. fluorescens* (78.26±10.68) and single inoculation of *G. mosseae* (46.2±13.05) for root colonization while *A. laevis*+ *P. fluorescens* (81.86±13.32) and *G. mosseae* (54.63±5.783) treatments were followed in case of AM spores number (Table 2). Among all bioinoculants, *G. mosseae* was found most efficient for both self proliferation as well as growth response.

Effects on biochemical parameters

Chlorophyll content: The photosynthetic pigments are important component of all autotrophs required in harbouring the light energy and then after its transformation to chemical energy utilised in metabolic assimilation. When the leaves of mycorrhizal plants were investigated for photosynthetic pigments (Table 3), it was found that plant inoculated with *G. mosseae*, *A. laevis* and *P. fluorescens* had maximum chlorophyll content (chl a- 0.32±0.03 mg. / gm. fresh wt., chl b- 0.76±0.17 mg. / gm. fresh wt. and total chl- 1.09±0.20 mg. / gm. fresh wt) than those plant inoculated with *A. laevis (*chl a- 0.05±0.00 mg. / gm. fresh wt., chl b- 0.09±0.01mg. / gm. fresh wt. and total chl- 0.15±0.01 mg. / gm. fresh wt*)* had minimum chlorophyll pigments among different treatments. The level of Chlorophyll A was recorded less in comparison to chlorophyll B in all treatments.

Phosphorus concentration and phosphatase activity: Different bio-inoculants manipulated the uptake of phosphorus to different extents although higher than control (Table 4). Maximum phosphorus content was found in case of plant inoculated with *G. mosseae* +*A. laevis* + *P. fluorescens* both in root **(**0.22±0.01 %) as well as shoot (0.16±0.09 %), results for minimum value obtained in treatment of *P. fluorescens* (root 0.11±0.03 % and shoot 0.11±0.04 %) but least content was observed in the control plant. The activity of enzyme more specifically phosphatase always confined to root in relation to better absorption of phosphorus from the soil. Better root architecture plays an influential role on phosphorus absorption.

Our investigation revealed that the experimental plants have maximum acidic phosphatase activity than alkaline; among different treatments, maximum enzymatic activities for both acidic (0.187±0.042 $IUg^{-1}FW$) & alkaline (0.079±0.030 $IUg^{-1}FW$) were reported in *G. mosseae*+ *A. laevis* + *P. fluorescens* treatment, while minimum phosphatase activity for both acidic (0.032±0.008 $IUg^{-1}FW$) and alkaline (0.019±0.002 $IUg^{-1}FW$) was found in plants augmented with *P. fluorescens*.

Flower yield: The yield expressed in term of total number of flower blossom per plant. Plants augmented with microbes showed significantly more flowering as compared to control (Table 3). Highest number of flowers was recorded in plant treated with *G. mosseae*+ *A. laevis* + *P. fluorescens* (9.00±2.00) which is further followed by *G. mosseae* (7.00±2.64) and *A. laevis (*5.00±1.73) as well as *G. mosseae* + *P. fluorescens* (5.00±1.00) treatment.

Plants also expressed variation in timing of emergence of flowering, among all treatments, *G. mosseae* shows early flowering in comparison to *G. mosseae*+ *A. laevis* + *P. fluorescens* treated plants**.**

Discussion

The productive improvement of plants through regular use of synthetic fertilizer is most common habit among farmer because nutrient availability is primarily important and yield determining factor.

Table 3
Interactive effect of AM fungi and *P. fluorescens* on Chlorophyll content and yield of *V. odorata* after 120 days

Parameters / Treatments	Chlorophyll content (mg. / gm. fresh wt.)			Yield (flower number)
	Chlorophyll a	Chlorophyll b	Total Chlorophyll	
Control	0.04±0.01d	0.07±0.01^{d}	0.12±0.01^{de}	2.00±0.00^{c}
Glomus mosseae	0.18±0.02b	0.23±0.13^{cd}	0.42±0.51^{cd}	7.00±2.64^{ab}
Acaulospora laevis	0.05±0.00d	0.09±0.01^{d}	0.15±0.01^{e}	5.00±1.73^{bc}
Pseudomonas fluorescens	0.16±0.04bc	0.31±0.09^{bcd}	0.47±0.12^{bc}	4.00±2.64^{bc}
A. laevis+ *G. mosseae*	0.13±0.00c	0.28±0.05^{bcd}	0.41±0.05^{cd}	4.00±2.64^{bc}
A. laevis + *P. fluorescens*	0.20±0.01b	0.45±0.11^{b}	0.66±0.12^{b}	4.00±1.73^{bc}
G. mosseae + *P. fluorescens*	0.18±0.00b	0.44±0.07^{bc}	0.63±0.07^{bc}	5.00±1.00^{bc}
G. mosseae+ *A. laevis* + *P. fluorescens*	0.32±0.03^{a}	0.76±0.17^{a}	1.09±0.20^{a}	9.00±2.00^{a}
L.S.D (P≤0.05)	00.038	0.202	00.224	3.461
ANNOVA F(7,16)	45.943	9.644	15.281	3.429

G†: *G. mosseae*, A: *A. laevis*, P: *P. fluorescens*, ‡: Each value is a mean of five replicates, ±: Standard deviation, AM: Arbuscular mycorrhizae, Values in columns followed by same letter are not significantly different, P≤0.05, least significant difference test

Table 4
Interactive effect of AM fungi and *P. fluorescens* on Phosphorus content and Phosphatase activity of *V. odorata* after 120 days

Parameters Treatments	Phosphorus content (%)		Phosphatase activity ($IUg^{-1}FW$)	
	Shoot	Root	Acidic	Alkaline
Control	0.06 ± 0.45^{b}	0.07 ± 0.21^{b}	0.026 ± 0.006^{d}	0.015 ± 0.004^{b}
Glomus mosseae	0.16 ± 0.01^{a}	0.14 ± 0.03^{a}	0.081 ± 0.035^{bc}	0.026 ± 0.012^{b}
Acaulospora laevis	0.16 ± 0.06^{a}	0.13 ± 0.01^{a}	0.069 ± 0.014^{c}	0.026 ± 0.006^{b}
Pseudomonas fluorescens	0.11 ± 0.04^{a}	0.11 ± 0.03^{a}	0.032 ± 0.008^{d}	0.019 ± 0.002^{b}
A. laevis+ G. mosseae	0.16 ± 0.06^{a}	0.18 ± 0.06^{a}	0.112 ± 0.016^{b}	0.028 ± 0.004^{b}
A. laevis + P. fluorescens	0.13 ± 0.02^{a}	0.19 ± 0.05^{a}	0.111 ± 0.005^{b}	0.022 ± 0.004^{b}
G. mosseae + P. fluorescens	0.15 ± 0.04^{a}	0.21 ± 0.02^{a}	0.114 ± 0.006^{b}	0.073 ± 0.045^{a}
G. mosseae+ A. laevis + P. fluorescens	0.16 ± 0.09^{a}	0.22 ± 0.01^{a}	0.187 ± 0.042^{a}	0.079 ± 0.030^{a}
L.S.D ($P\leq0.05$)	0.292	0.145	0.346	0.039
ANNOVA F(7,16)	2.812	15.97	22.81	3.69

G†: *G. mosseae*, A: *A.laevis*, P: *P. fluorescens*, ‡: Each value is a mean of five replicates, ±: Standard deviation, AM: Arbuscular mycorrhizae, Values in columns followed by same letter are not significantly different, $P\leq0.05$, least significant difference test

But huge application of synthetic fertilizers leads to degradation of long term soil fertility, pollution and increases disease susceptibility in plant. The use of soil microbes as bio-fertilizer due to their ability to provide nutrients to plant acts as an eco-friendly substitute in place of synthetic fertiliser, the beneficial mycorrhizal fungi not only enhance plant growth but also self proliferates under the influence of the host due to their obligate symbiotic behaviour[28].

Inoculation of *V. odorata* with AMF and *P. fluorescens* increased plant growth (shoot height, root length, fresh and dry biomass) over untreated control plants. A significant correlation was noticed in vegetative parameters like crown size, dry weight of shoot, leaf area and leaf number with mycorrhization parameters. Hemavathi et al[29] reported growth enhancement in *Ocimum basilicum* that was inoculated with *G. fasciculatum* and PGPR. Positive impact of mycorrhizal fungi on growth of *Zingiber officinale* grown under pot conditions has been confirmed by Samanhudi et al[30].

Similarly, Vafadar et al[31] recorded profound effects of dual inoculation of *Azotobacter chroococcum* and *G. intraradices* on shoot height, root length, root and shoot biomasses as well as absorption of nitrogen, phosphorus and potassium in *Stevia rebaudiana* after 60 days of growth period. Improved root architecture due to emergence of extra radical hyphae results in increase of root surface area of host plant for efficient absorption of water, macro and micro nutrients from depleted zone of soil as compared to control plants.
Amaya Carpio et al[32] confirmed that an increased nutrient absorption capability of inoculated plant tends to improve their leaf area and photosynthesis. Increased leaf area in AM inoculated plant might correspond to improved nitrogen fixation, synthesis of amino acid, synthesis of carbohydrates and organic acids[33,34]. AMF and *P. fluorescens* inoculation show synergistic effects on yield parameter i.e. flower number and correspond to better vegetative growth which is accompanied by high rate of photosynthesis.

Saini et al[35] conducted a field experiment to study the efficacy of bioinoculants like *G. mosseae, A. laevis, Trichoderma viride, P. fluorescens* on growth parameters of *Zinnia elegans* and found increase in growth parameters, nutrient assimilation and yield due to increased transformation of native nutrients such as phosphorus, zinc, copper, iron and sulphur from fixed to soluble form. Moreover, flower yield of the plant might be associated with increased production of growth hormones such as auxin and gibberellins due to soil microbes having fertilizer potential that induce bud production due to increased nutrient absorption more specifically potassium by the plant[36,37].

Both Gaur and Adholeya[38] and Aboul-Nasr[39] have reported an increase in flower number when angiospermic plants were inoculation with Arbuscular Mycorrhizal fungi. The mycorrhizal plants also showed early and vigorous flowering as compared to non-mycorrhizal plant which results into increased flower dry weight. An early flower induction has been reported in *Rosa hybrid by* Garmendia and Mangas[40] and an increased flower dry weight was reported by Shamshiri et al[41]while working on *Petunia* sp.

Inoculation of plants with consortium of *G. mosseae, A. laevis, P. fluorescens* resulted in highest sporulation and percent root colonization that might be due to synergistic interaction between *P. fluorescens* and AM fungi. Increased mycorrhization was directly related to symbiotic intimacy between fungus and host plant and leads to exchange of nutrients. However, extent of host plant mycorrhization is majorly restricted by availability of phosphate in soil. High

phosphate concentration in the soil adversely affects biofertilizer potential of AM fungi through controlling spore germination, carbon supply to the developing hyphae and growth of emerging hyphae from germinating spores[42].

Both AMF and phosphate solubilizing bacteria (PSB) inoculated plants had dramatically higher level of total chlorophyll content that might be attributed by increased level of magnesium, nitrogen and phosphorus than single inoculation[43,44]. In same way, Babaei et al[45] demonstrated a positive relationship between AMF and PSB and also reported maximum nutrient acquisition in inoculated plants of *Helianthus annuus.* Better nutritional conditions are conducive to significant improvement in ability to synthesize chlorophyll and accessory pigments which helps to convert photonic energy into chemical energy and thus leads to better growth[46].

Similar results of significant increase in chlorophyll were reported by Adolfsson et al[47] while studying the resilience of *Jacaranda mimosifolia* in urban areas on account of mycorrhization[48]. Our results are in accordance with Vafadar et al[31] who reported that dual inoculation of AM fungi and N-fixing bacteria is an effective treatment to improve total chlorophyll content in *Stevia robusta.*

In present investigation, both alkaline and acidic phosphatase activity were found improved in all treatment except control one, acidic phosphatase activity is more profound than alkaline and progressively enhanced with both plant growth as well as mycorrhization status. The acidic phosphatase activity may be linked with proper establishment and growth of mycorrhizal hyphae inside the host tissue and also associated with better acquisition of phosphorous from rhizo-sphere[49]. The acidic phosphatase activity is usually concerned with increased uptake of phosphorus from the rhizospheric soil of plant while alkaline phosphorus activity may be involved in active transport of phosphate to the AM colonized roots[50].

Our findings are in accordance with Tanwar et al[51] who reported improved nutrient uptake as well as increment in enzymatic activity concerned to mineralized immobile phosphorus in broccoli with AM fungi. Similarly, Jangra et al[52] recorded maximum phosphatase activity in *Murraya koenigii* L plant inoculated with a consortium of AMF and phosphate solubilising bacteria.

The experimental analysis revealed that the phosphorus uptake in viola leaf tissue was more profound in mycorrhizal plant than control and positively linked with application of an efficient AM species either independently or in combination with phosphate solubilising bacteria.

According to George et al[53], an increased phosphorus uptake in mycorrhizal plant occurred due to the exploration ability of arbuscular mycorrhizal fungal hyphae to trace more soil volume in search of nutrients beyond the nutrient deficient zone and act as a channel for transport of phosphorus from the soil to plant root. A significant increase in percentage of root and shoot P in *Zinnia elegans* was also observed by Saini et al[35] when inoculated with AM fungi either independently or in combination with other bio-inoculants like *P. fluorescens* and *Trichoderma viride*.

Conclusion

Arbuscular mycorrhizal fungi provide benefits to the host plants symbiotically in many aspect such as crown size of shoot, number of leaves, total leaf area, shoot and root biomass, the uptake of phosphorus and other nutrients, number of flowers, level of photosynthetic pigments and phosphatase activity of roots. These benefits could be attributed to the increased surface area of roots, enhanced uptake of nutrients, better water absorption, secretion of some enzymes and organic acids by inoculated microorganisms.

Among different treatments, a consortium of *Glomus mosseae*, *Acaulospora laevis* and *Pseudomonas fluorescens* was found best combination while *G. mosseae* as most efficient strain. Keeping in view the results of above controlled studies, the field trial is further needed to access the synergistic and symbiotic efficacy of bio-inoculants in growth promoting aspects under natural conditions where climatic factors and microbes have to intimate with each other to give favourable outcomes. After that these bio – inoculants can be utilized in large scale cultivation of *V. odorata* for improvement in plant biomass as well as yield.

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