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ROLE OF PLANT GROWTH PROMOTING RHIZOBACTERIA IN THE PROLIFERATION AND BIOCONTROL ACTIVITY OF VESICULAR ARBUSCULAR MYCORRHIZAE (VAM) IN SOIL

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ABSTRACT

Symbiotic relationship of Arbuscular mycorrhizal fungi with plants reported for a long time. AMF regulates growth and development of plants by colonizing under the cortical region of roots and promotes nutrient uptake especially phosphorus (P) and nitrogen (N) to plants. Spores of VAM are isolated from mycorrhizospheric soil. Among identified Vesicular arbuscular mycorrhiza (VAM) fungi, *Glomus* was found dominant, however, *Acaulospora* and *Gigaspora* were also recorded. For the establishment of intra radical colonization and proliferation of VAM fungi, endophytic fluorescent *Pseudomonas* (PGPR) strains were applied and indicated the biocontrol ability of VAM fungi with the induction of systemic resistance against root rotting pathogens. Six endophytic fluorescent pseudomonas (EFP) isolates of EFP-102, EFP-105, EFP-106, EFP-110, EFP-111 and EFP-112 were obtained from Agriculture and Biotechnology Laboratory Culture Collection Centre, and tested *in vitro* against four root decaying fungi *viz*., *Rhizoctonia solani, Fusarium oxysporum*, *F. solani* and *Macrophomina phaseolina.* In dual plate culture assays Plant growth promoting rhizobacteria (PGPR) showed significant suppressive effect on root rot fungi. Culture filtrates of four screened strains of EFP tested by disc diffusion method against root rot. *In vivo* combined treatment of EFP isolates and VAM spores under screen house in sterilized soil showed improvement in plant growth and yield. Sterilized soil make a good impact on proliferation of VAM spores. EFP isolates, improves phosphorus and nitrogen uptake and upgrades systemic resistance.

Keywords: Vesicular arbuscular mycorrhizae, fluorescent *Pseudomonas,* VAM spores, Rhizospheric soil

INTRODUCTION

Symbiotic relationship of AMF with plants reported for a long time (Selosse *et al.,* 2015). AMF regulates growth and development of plant by colonizing mycelium under the cortical region of roots and promotes nutrient uptake especially nitrogen (N) and phosphorus (P) to plants (Smith and Read, 2008) sometime it might be happened that they belong to different species may form a common mycorrhizal network (CMN) (Pringle e*t al.,* 2009). Soil characteristics also improved by several changes in their morpho-physiological traits (Hashem *et al.,* 2015) that encouraged plant development in both normal and in stressful conditions (Navarro *et al.,* 2014). Additionally, soil with AMF-inoculation has more persistent masses and contains high extra radical hyphae (Syamsiyah *et al.,* 2018). Functions that depend on growth, for example, stomatal conductance, relative water content (RWC), the water potential of a leaf, PSII efficiency and CO_2 absorption are also affected by AMF inoculation (Chandrasekaran *et al.,* 2019). Plant body requires mineral elements, such as N, P and K for their growth. Numerous sugar-phosphate, nucleic acids, and phospholipids are made up of phosphorus (Taiz and Zeiger, 2002) which is not readily available for plants involved in photosynthesis, growth and development. Deficiency of inorganic phosphate (Pi) lead yield losses in many cereals (Balemi and Negisho, 2012). P absorption is the major benefit of the VAM symbiosis for plants (Riaz *et al.,* 2007).Nitrogen is abundant for proper plant growth and development with enhancing yield and quality (Leghari, 2016) absorbed in the form of NO_3^- and $NH_4^+$from the soil (Xu *et al.,* 2012). AMF fungi also have a high ability to degrade polymeric N for the amino acid absorption (Smith and Read, 2007) as compared to the Non-AMF plants (Näsholm *et al.,* 2009).

Phytochemicals like Phenols take part in plant resistance, regulation of seed germination and defense mechanism during biotic and abiotic stress (Kubalt, 2016). Salicylic acid (SA) is naturally involved in the improvement of systemic acquired resistance (SAR) (Farhat *et al.,* 2023) against several nematodes, insect pests, bacterial and fungal infections. Among the group of plant growth-promoting rhizobacteria (PGPR), fluorescent *Pseudomonas* are considered more powerful agents for improving soil quality, plant growth and suppression of root pathogens (Urooj *et al.,* 2021) by producing different types of antibiotics and siderophores against pathogens (Noreen *et al.,*2015). These all compounds are key for inducing resistance against several diseases (Rahman *et al.,* 2017). The mixed

application of VAM with certain PGPR has been reported to increase the colonization and proliferation of VAM on plant growth (Sumana *et al.,* 2003). Several researches showed combinations of various bacteria and fungi in biocontrol activities as compared to the microbes used singly (Xu *et al.,* 2011). *Pseudomonas* spp. that act as mycorrhizal-helper bacteria showed positive effect on yields of wheat as compared to non-treated with high soluble P fertilizer and also enhanced biocontrol activity (Guetsky *et al.,* 2001). There is a need to investigate new bacterial helpers of fungi and the mechanisms involved in this interaction for efficient development. Bacteria belonging to the genus *Rhodanobacter* (Xanthomonadales) appeared in determining communities of AMF (Svenningsen *et al.,* 2018) and these considered as fungiphile (Simon *et al.,* 2015). The root diseases of sunflower and mungbean were significantly suppressed by the application of mycorrhizospheric fluorescent *Pseudomonas (*MRFP) which enhanced mycorrhizal population and improved phosphorus uptake in plants both in screen house and field experiments (Bokhari *et al.,* 2013). The intra radical colonization of *Glomus intraradices* on chickpea improved by endophytic *Pseudomonas putida* and *Paenibacillus polymyxa* (Akhtar and Siddiqui, 2008). The effect of combined application on tomato by *G. mosseae* and *Pseudomonas fluorescens* significantly suppressed the infection of *Rhizoctonia solani* (Berta *et al.,* 2005). PGPR facilitated colonization by VAM fungi, improved the development of the mycosymbiont and improved phosphorus uptake by VAM fungi (Ehteshamul-Haque *et al.,* 2015). *P. fluorescens* with *G. mosseae* significantly reduced root galling by *Meloidogyne javanica* as compared to control (Siddiqui and Mahmood, 2001). In the present study six antagonistic strains of endophytic fluorescent *Pseudomonas* (EFP-102, EFP-105, EFP-106, EFP-110, EFP-111 and EFP-112) were used as mycorrhizal helper bacteria for the evaluation of their role in the proliferation of VAM fungi and inhibition of root diseases by suppressing root rot pathogens such as *Rhizoctonia solani*, *Fusarium oxysporum*, *F. solani* and *Macrophomina phaseolina*. The present research also focused on the combined effect of EFP and VAM spores in the establishment and proliferation of VAM fungi on mung beans, used as test plants and quantitative estimation of intra radical colonization of VAM.

MATERIALS AND METHODS

Endophytic Fluorescent Pseudomonas

For this study, endophytic fluorescent *Pseudomonas* strains (EFP-102, EFP-105, EFP-106, EFP-110, EFP-111 and EFP-112) was obtained from Agriculture and Biotechnology Laboratory Culture Collection Centre, Department of Botany, University of Karachi, as these strains in another study showed good results against common laboratory bacteria. These strains were originally isolated by Bukhari *et al.* (2023).

Quantitative estimation of VAM spores from soils

Decanting and Wet sieving technique used for the extraction of VAM spores from soil and soils were collected from different rhizospheric region of plants viz., *Abelmoschus esculentus*, *Capsicum frutescens*, *Cenchrus ciliaris*, *Lagenaria siceraria, Solanum lycopersicum*, *Spinacia oleracea*, *Solanum melongena*, *Triticum aestivum*, *Vigna radiate*, *Zea mays*, where soil was air-dried, mixed (100 g) in water (1 L), left for 20 min and passed through a series of sieves of 400, 200, 150 and 53 µm (descending order). Residues left on sieves (150 and 53 µm) were collected in 100 mL beakers and used for the examination and quantitative estimation of VAM spores under stereo binocular microscope (Bukhari *et al.,* 2023).

Preparation of slides of VAM spores

For semi-permanent mountant a drop of Poly Vinyl Lectophenol (PVL) or Poly Vinyl Lecto-Glycerol (PVLG), which allowed microscopic slides to remain usable for years. VAM spores were placed on a glass slide and a clean dry coverslip was placed and excess mountant was removed with a cotton swab. Cover slip was sealed and VAM spores were identified on the basis of diagnostic characteristics like color, size, spore wall and type of hyphal attachment (Schenck and Perez, 1990).

In vitro test against root infecting fungi by dual culture plate assay method

For determination of fungicidal potential 06 isolates of endophytic fluorescent *Pseudomonas* strains (EFP-102, EFP-105, EFP-106, EFP-110, EFP-111 and EFP-112) were tested against four root rotting fungi initially isolated from culture collection center of Karachi University, *viz*., *Fusarium oxysporum*, *F. solani, Macrophomina phaseolina* and *Rhizoctonia solani*. A 5 mm disc of root rotting fungi inoculated on one side of the Petri dish and streaked antagonistic bacteria on the other side of CDA (Czapek's Dox Agar) containing Petri plates and incubated at 28°Cfor 1 week. Inhibition zones were calculated according to Korejo *et al.* (2014).

Culture filtrates of endophytic fluorescent Pseudomonas EFP

Test isolates of EFP were grown in KB broth at 30°C for 3 days, then centrifuged at 3000 rpm for 20 minutes. Then 1-2 drops of chloroform were added to prevent further growth of any contaminant. Sterility was checked by spreading them on King's B medium.

Antifungal activity of culture filtrates of EFP

Thick sterile filter paper discs (5 mm) were loaded with culture filtrates at 20, 40 and 60 µL per disc and placed in clockwise manner on petri plates containing Czapek's Dox Agar. A 5 mm disc of root rotting fungi was inoculated in the center of the plates. Topsin (20 µg/disc) served as +ve control, while sterilized broth served as a negative control. Zone of inhibition was recorded after 5 days growth (Farhat *et al.,* 2019; 2022).

***In vivo* screen house experiments**

In vivo, effects of endophytic fluorescent *Pseudomonas* strains on plant growth potential were evaluated for their biocontrol activities against root rot fungi; *Fusarium oxysporum*, *F. solani, Macrophomina phaseolina* and *Rhizoctonia solani* and also to check the plant biomass. Aqueous suspension (10^7 CFU/mL) of *Pseudomonas*s trains (EFP-102, EFP-105, EFP-106, EFP-112 were drenched in experiments separately in order to check the efficacy of *Pseudomonas* strains while in the other set combine application of *Pseudomonas* with VAM (2-5 spores/g) were applied. The application of *Pseudomonas* strains and VAM were collectively applied (EFP-102+VAM, EFP-105+VAM, EFP-106+VAM, EFP-112+VAM). In each pot 6 mung bean seeds were sown, and upon germination 4 seedings were left and the rest of them were removed. Observations were recorded on plant growth after 45 days. Experiments were conducted in sterilized soil (Bukhari *et al.,* 2023).

Growth parameters

Growth parameters were taken after uprooting of the plants including weight and length of roots and shoots; number of pods and nodules formed.

Infection of root rotting Fungi

After 45 days of growth, the plants physical parameters were taken and their roots are selected to check the infection percentage of root rot pathogens. The infected roots were washed under the tap water, cut into 1 cm pieces and sterilized with 1% $Ca(OCl)_2$ and transferred onto Petri dishes containing Potato Dextrose Agar. Penicillin (100000 units/L) and Streptomycin (0.2g/L) were added in the medium to prevent the bacterial growth. Fungi emerged from root pieces after 5 day incubation and were identified and infection % were calculated (Farhat *et al.,* 2023).

Assessment of root length colonization of VAM Fungi

Using Biermann and Linderman, (1981) technique, mycorrhizal colonization in plant roots is assessed. For this purpose, roots are thoroughly washed with KOH (10%) and H_2O_2 (10%) and stained with 0.05% Trypan blue in lacto phenol. The fungal structures present within the roots were observed under the microscope (400X).

Quantitative estimation of intraradical colonization of VAM Fungi

Using Biermann and Linderman, (1981) method (frequency distribution method) the quantitative estimation of vesicles present in plant roots was performed as number in 100 g dry soil.

Biochemical parameters

Estimation of Phosphorus

Dry ashing method was used for the assessment of phosphorus content in leaves. The Barton reagent was used to estimate total phosphorus content by having 0.2 g of oven dried powdered leaf, digested in 2 mL H_2SO_4 (conc.) to which 30% solution of H_2O_2 drop wise poured until a colorless solution appeared at the bottom of the flask. It is repeated until and unless 1-2 mL of digested sample is left. Final volume was adjusted up to 100 mL with distilled water and filtered (10 mL) of digested sample was added in addition to 10 mL Barton reagent. The prepared sample then incubates for 30 min at room temperature. Absorbance was recorded on a spectrophotometer at 420 nm. Standard curve of KH_2PO_4 is used for the estimation of phosphorus (Rayan *et al.,* 2001).

Phosphorus (ppm) = Phosphorus value (ppm) from standard curve × Total dilution factor (T.D.F)

Estimation of Nitrogen

Nitrogen estimation is done by Nessler's method in plants (Singh, 1982). For this purpose digested sample used same as phosphorus estimation, 01 mL of digested sample was added in a 50mL flask along with 01 mL NaOH (10%), 01 mL of Na-silicate (10%) with 15 mL of Nessler's reagent. The final volume was maintained up to 50 mL using distilled water. The prepared sample was incubated for 20 min at room temperature. The absorbance is measured on a spectrophotometer at 410 nm. Standard curve of NH_4NO_3 is used for the estimation of Nitrogen.

Nitrogen (ppm) = Nitrogen value (ppm) from standard curve × Total dilution factor (T.D.F)

Estimation of total polyphenol and salicylic acid

Oven dried leaves were crushed in ethanol (96% v/v) and then centrifuged at 3000 rpm for about 20 min. Total polyphenols were determined by using reagent of Folin-Ciocalteu phenol described by (Chandini *et al.,* 2008), add 2% Na_2CO_3 2mL collected and mixed up in 100µL aliquot of 10mg/mL extracts and then left for about 2 min at room temperature. 100µL of (50%) Folin-Ciocalteu Reagent also added and then mixed well and it was put in dark for about 30 minutes. Absorbance was read at 270nm. Standard curve of Gallic acid was used to determine total phenolic contents**.**

Sample was prepared the same as total polyphenols. According to the method described by Warrier *et al.*, (2013) aliquots (0.1mL) mixed with freshly prepared (0.1%) solution of ferric chloride. Each sample was brought up to (3.0 mL) and absorbance was read at 530nm on a spectrophotometer.

Data analysis

Statistical software, CoStat, CA, USA and SPSS were used for the analysis of variance (ANOVA), then means were separated and least significant difference (LSD) and Duncan's multiple range test was calculated.

RESULTS

Isolation of VAM spores from different regions of cultivation

VAM spores were isolated from mycorrhizospheric soil of 20 healthy plants including *Abelmoschus esculentus*, *Capsicum frutescens, Cenchrus ciliaris, Helianthus annuus, Lagenaria siceraria, Triticum aestivum*, *Trigonella* sp., *Solanum melongena, S. lycopersicum, Spinacia oleracea, Vigna radiata,* and *Zea mays* from different regions of Jamshoro, Karachi University, Malir and Mirpurkhas. VAM spores were identified by their diagnostic characteristics like color, size, spore wall and types of hyphal attachments. Among these, the genus *Glomus* was found dominant, however, *Acaulospora* and *Gigaspora* were also associated with the mycorrhizospheric region of plants. The highest population of spores per gram of soil shown in *A. esculentus* whereas lowest population recorded in soil of *Lagenaria siceraria* (Table 1).

In vitro antifungal activity of endophytic fluorescent Pseudomonas (EFP) isolates

Fungicidal potential of endophytic fluorescent *Pseudomonas* isolates were tested against root rot pathogens by dual culture plate assay on Czapek's Dox Agar. All isolates showed suppressive impacts on all four tested fungi *viz*., *F. oxysporum, F. solani, M. phaseolina* and *R. solani*. The maximum zones of inhibition shown by EFP against root rot fungi up to 30mm (Table 2).

In vitro antifungal activity of culture filtrates of EFP by disc diffusion method

The antifungal activity was determined by agar disc diffusion method of culture filtrates of EFP isolates (EFP-102, EFP-105, EFP-106 and EFP-112) against *M. phaseolina*, *F. oxysporum*, *F. solani* and *R. solani*. Culture filtrates of all four endophytic fluorescent *Pseudomonas* produced major zones of inhibitions at 60 µL per disc against root rotting fungi (Table 3).

Application of endophytic fluorescent Pseudomonas and VAM spores

Four isolates of EFP that showed strong activities *in vitro* were further subjected for *in vivo* study. *In vivo* pot experiments were conducted in screen houses under the same environmental conditions. Sterilized soil used for each experiment. The host plant selected was leguminous plant *Vigna radiata* (mungbean).Combined effects of endophytic fluorescent *Pseudomonas* in the establishment and proliferation of VAM fungi, phosphorus and nitrogen uptake by plants were also evaluated. The effect of EFP was also compared with non-inoculated EFP plants which served as control.

Combined effects of EFP and VAM spores on infection of root rotting fungi

In this set of experiment autoclaved (garden soil autoclaved twice for 45-50 minutes at 15 lb.) At 45 days, plants treated alone or combined with endophytic fluorescent *Pseudomonas* isolates (EFP-102, EFP-105, EFP-106 and EFP-112) and VAM spores showed positive suppression of pathogenic fungi viz *M. phaseolina, F. oxysporum, F. solani* and *R. solani* as compared with non-treated plants. Infection of *F. oxysporum* was reduced from 75% (control) to 12.5% same as treated with VAM spores and EFP-105 and completely suppressed 0 % (treated combined EFP-105 with VAM spores) showed significant difference between treatments. In case of *F. solani*, incidence of infection reduced from 75% (control) to 18.7 % (VAM) and same results appeared 6.2% when plants treated with EFP-106 and combined with VAM spores but complete suppression of fungal hyphae was seen in application of EFP-112+VAM spores, whereas 68.7% (control) infection of *M. phaseolina* controlled by VAM spores (12.5%) while combine application of EFP-105, EFP-106 and EFP-112 showed same results (6.2%). Infection of *R.solani* was suppressed by inoculation of aqueous suspension of EFP-105 with VAM spore (0 %) completely as compared to the control 81%. However Topsin which served as positive control became less effective against all root rotting fungi as compared to alone and combined application of EFP and VAM spores. The *Pseudomonas* isolates such as EFP-105 and EFP-112 showed effectiveness in controlling root infection very well (Table 4).

Table 1. VAM spores per gram of soil from different locations of Sindh.

Plants sources	Spores	VAM Fungi	Locality
1. *Abelmoschus esculentus*	36	*Glomus mossae, Acaulospora* sp., *Glomus* sp.	M
2. *Abelmoschus esculentus*	36	*Glomus* spp., *Glomus mossae*	KU
3. *Abelmoschus esculentus*	36	*Glomus* spp., *Glomus mossae*	MR
4. *Capsicum frutescens*	23	*Glomus mossae*	KU
5. *Capsicum frutescens*	18	*Glomus* spp., *Gigaspora* spp.	KU
6. *Capsicum frutescens*	23	*Glomus fasiculatum*, *Glomus mossae*	J
7. *Capsicum frutescens*	23	*Glomus mossae, Gigaspora* spp., *Glomus* spp.	M
8. *Cenchrus ciliaris*	34	*Glomus* spp.	M
9. *Helianthus* spp.	26	*Glomus* spp., *Gigaspora* sp.	KU
10. *Lagenaria siceraria*	20	*Glomus* spp., *Gigaspora* spp.	J
11. *Lagenaria siceraria*	17	*Acaulospora* sp., *Glomus* spp.	MR
12. *Solanum lycopersicum*	27	*Glomus fasiculatum, Glomus* spp.	KU
13. *Solanum melongena*	27	*Glomus* spp., *Glomus mossae*	J
14. *Solanum melongena*	31	*Glomus* spp., *Gigaspora* spp.	KU
15. *Spinacia oleracea*	19	*Acaulospora* sp., *Glomus* spp.	MR
16. *Spinacia oleracea*	19	*Glomus* sp. *Gigaspora* sp.	M
17. *Trigonella* sp.	22	*Glomus* spp.	M
18. *Triticum aestivum*	30	*Glomus mossae, Acaulospora* sp.	M
19. *Vigna radiata*	21	*Glomus fasiculatum, G mossae*	KU
20. *Zea mays*	29	*Glomus fasiculatum*, *Glomus* spp.	M

KU= Karachi University field, M= Malir, MR=Mirpurkhas, J=Jamshoro

Table 2. *In vitro* growth inhibition of *Fusarium oxysporum, F. solani*, *Macrophomina phaseolina* and *Rhizoctonia solani* by endophytic fluorescent *Pseudomonas* isolates.

Pseudomonas isolates	*F. oxysporum*	*F. solani*	*M. phaseolina*	*R. solani*
	Zones of inhibition(mm)			
EFP-102	$26^{ab} \pm 2.8$	$25^{cd} \pm 2.6$	$8^{a} \pm 1.2$	$12^{b} \pm 1.2$
EFP-105	$28^{bc} \pm 3.1$	$30^{de} \pm 3.0$	$26^{bc} \pm 2.7$	$28^{bc} \pm 2.9$
EPF-106	$28^{bc} \pm 3.0$	$19^{bc} \pm 1.6$	$0^{a} \pm 0.0$	$11^{a} \pm 1.1$
EFP-110	$19^{a} \pm 1.6$	$25^{c} \pm 2.6$	$3^{a} \pm 0.1$	$4^{a} \pm 0.2$
EFP-111	$19^{a} \pm 1.6$	$21^{b} \pm 2.2$	$6^{a} \pm 0.7$	$6^{a} \pm 0.7$
EFP-112	$20^{bc} \pm 2.0$	$30^{d} \pm 2.6$	$11^{ab} \pm 1.1$	$18^{cd} \pm 1.5$

Similar letter are not significantly different from each other according to DMRT at $p<0.05$.

Table 3. *In vitro* antifungal activities of cell free culture filtrates of endophytic fluorescent *Pseudomonas* (EFP).

Pseudomonas isolates	Treatments	*F. oxysporum*	*F. solani*	*M. phaseolina*	*R. solani*
	Zones of inhibition(mm)				
	Control (negative)	$0^{a} \pm 0.0$	$0^{a} \pm 0.0$	$0^{a} \pm 0.0$	$0^{a} \pm 0.0$
EFP-102	20 µL/disc	$1^{ab} \pm 0.8$	$0^{a} \pm 0.0$	$0^{a} \pm 0.0$	$0^{a} \pm 0.0$
	40 µL/disc	$6^{bc} \pm 2.1$	$1^{ab} \pm 0.9$	$2^{ab} \pm 1.4$	$2^{ab} \pm 1.1$
	60 µL/disc	$8.5^{cd} \pm 3.4$	$7^{b} \pm 3.3$	$8^{b} \pm 2.9$	$10^{bc} \pm 3.6$
EFP-105	20 µL/disc	$0^{a} \pm 0.0$	$0^{a} \pm 0.0$	$1.4^{ab} \pm 2.0$	$0^{a} \pm 0.0$
	40 µL/disc	$2^{ab} \pm 1.2$	$3^{ab} \pm 1.7$	$6^{b} \pm 3.9$	$2^{b} \pm 1.1$
	60 µL/disc	$6^{b} \pm 3.5$	$8^{b} \pm 3.8$	$11^{c} \pm 4.7$	$12^{c} \pm 3.0$
EFP-106	20 µL/disc	$1^{a} \pm 1.0$	$0^{a} \pm 0.0$	$3^{ab} \pm 2.0$	$0^{a} \pm 0.0$
	40 µL/disc	$4^{a} \pm 1.9$	$3^{ab} \pm 2.8$	$4.2^{ab} \pm 3.1$	$2^{ab} \pm 1.4$
	60 µL/disc	$11^{cd} \pm 2.7$	$10.1^{d} \pm 3.0$	$7.5^{d} \pm 4.1$	$10^{cd} \pm 3.8$
EPF-112	20 µL/disc	$1^{a} \pm 0.5$	$1^{a} \pm 0.9$	$0^{a} \pm 0.0$	$0^{a} \pm 0.0$
	40 µL/disc	$2.5^{ab} \pm 3.6$	$7^{b} \pm 2.5$	$6^{bc} \pm 1.8$	$4^{b} \pm 1.6$
	60 µL/disc	$8.5^{d} \pm 4.1$	$11^{de} \pm 3.7$	$10.2^{d} \pm 4.6$	$9.4^{cd} \pm 3.9$

Values in column bearing same superscript letter are not significantly different at $p<0.05$ according to Duncan's multiple range test.

Table 4. Effect of EFP on the infection of *F. oxysporum, F. solani, M. phaseolina* and *R. solani* under screen house experiment in sterilized soil.

	Treatments	*F. oxysporum*	*F. solani*	*M. phaseolina*	*R. solan*
			Infection %		
1.	Control	75	75	68.7	81.2
2.	Topsin5	6.2*	50*	56.2*	50*
3.	VAM spores	12.5*	18.7*	12.5*	6.2*
4.	EFP-102	25*	18.7*	12.5*	12.5*
5.	EFP-105	12.5*	12.5*	25*	6.2*
6.	EFP-106	18.7*	6.2*	18.5*	18.5*
7.	EFP-112	12.5*	12.5*	8.5*	6.2*
8.	EFP-102+VAM	6.2*	6.2*	12.5*	12.5*
9.	EFP-105+VAM	0*	6.2*	6.2*	0*
10.	EFP-106+VAM	6.2*	6.2*	6.2*	6.2*
11.	EFP-112+VAM	6.2*	0*	6.2*	6.2*
$LSD_{0.05}$	**Treatment=7.07[1],**			**Pathogen=4.26[2]**	

[a]In column, mean values showing differences more than LSD value are significantly different at $p < 0.05$
[b]In row, mean values showing differences more than LSD value are significantly different at $p < 0.05$
*Significant at $p < 0.05$ as compared to untreated control

Impact of EFP and VAM fungi in salicylic acid and total polyphenols

In sterilized soil, concentration of total polyphenol increased from lowest (0.06 ± 0.00^{a} µg/mL) in control to highest (0.17 ± 0.00^{g} µg/mL) in EFP-106+VAM. Application of positive control Topsin, EFP-106 and EFP-112 showed similar effects that is 0.09 ± 0.00^{c} µg/mL while application of VAM spores, mixed application of EFP-105+VAM and EFP-112+VAM also showed similar effects that is 0.15 ± 0.00^{e} µg/mL. In sterilized soil, concentration of salicylic acid showed a positive impact when EFP inoculated either alone or with VAM spores. Concentration of salicylic acid raised from 0.16 ± 0.00^{a} µg/mL (control) up to 0.43 ± 0.00^{d} µg/mL (EFP-

112+VAM). Concentrations obtained by the application of VAM spores, EFP-102, EFP-105, EFP-106, EFP-112 and mixed inoculation of EFP-105+VAM showed almost similar effects (Fig. 3).

Table 5. Effect of EFP and VAM spores on growth parameters of mungbean and in the establishment and proliferation of VAM fungi in sterilized.

Treatments	Shoot length (cm)	Root length (cm)	Shoot weight (g)	Root weight (g)	numbers of nodules/plant	numbers of pods	spores/gram in soil	numbers of vesicles/cm in root
Control	12.55 ± 1.08^{a}	11.50 ± 0.45^{a}	1.3 ± 0.24^{a}	0.19 ± 0.009^{a}	1.00 ± 0.12^{a}	1.00 ± 0.12^{a}	3.00 ± 0.14^{a}	2.00 ± 0.34^{a}
Topsin	17.65 ± 1.22^{b}	9.90 ± 2.81^{a}	2.27 ± 0.26^{bc}	0.24 ± 0.03^{ab}	5.00 ± 0.56^{d}	2.00 ± 0.31^{bc}	3.00 ± 0.13^{a}	2.00 ± 0.64^{a}
VAM	20.37 ± 1.586^{cd}	21.82 ± 1.17^{c}	2.69 ± 0.25^{cd}	0.41 ± 0.15^{abcd}	5.00 ± 0.3^{bcd}	1.00 ± 0.45^{bcd}	10.0 ± 0.66^{d}	7.00 ± 0.63^{e}
EFP-102	20.14 ± 2.10^{cd}	12.76 ± 3.13^{a}	2.48 ± 0.47^{c}	0.33 ± 0.05^{abc}	7.00 ± 0.61^{e}	1.00 ± 0.55^{ab}	4.00 ± 0.66^{a}	3.00 ± 0.30^{ab}
EFP-105	19.87 ± 2.06^{cd}	12.07 ± 2.28^{a}	2.04 ± 0.12^{bc}	0.27 ± 0.03^{abc}	8.00 ± 0.35^{f}	2.00 ± 0.38^{cd}	3.00 ± 0.69^{a}	$3.00 \pm .61^{bc}$
EFP-106	20.25 ± 1.78^{cd}	11.30 ± 2.96^{a}	1.80 ± 0.40^{ab}	0.44 ± 0.10^{abc}	4.00 ± 0.49^{cd}	1.00 ± 0.65^{abc}	3.00 ± 0.39^{a}	2.00 ± 0.38^{ab}
EFP-112	18.75 ± 0.86^{bc}	12.35 ± 1.67^{a}	1.75 ± 0.32^{ab}	0.54 ± 0.14^{cd}	5.00 ± 0.73^{d}	2.00 ± 0.37^{bcd}	3.00 ± 0.55^{a}	4.00 ± 0.80^{c}
EFP-102+VAM	18.87 ± 0.85^{bc}	17.12 ± 0.85^{b}	3.30 ± 0.46^{d}	0.56 ± 0.09^{e}	4.00 ± 0.36^{c}	2.00 ± 0.67^{bcd}	8.00 ± 4.00^{bc}	4.00 ± 0.29^{c}
EFP-105+VAM	21.15 ± 0.91^{d}	15.82 ± 2.38^{b}	3.27 ± 0.71^{d}	0.51 ± 0.13^{bcd}	3.00 ± 0.62^{b}	2.00 ± 0.20^{d}	6.00 ± 2.45^{b}	4.00 ± 0.53^{c}
EFP-106+VAM	25.90 ± 0.66^{e}	20.50 ± 1.95^{c}	4.32 ± 0.20^{e}	1.07 ± 0.22^{e}	5.00 ± 0.45^{cd}	4.00 ± 0.45^{e}	8.00 ± 0.12^{c}	5.00 ± 0.32^{d}
EFP-112+VAM	26.07 ± 0.94^{e}	17.27 ± 1.35^{b}	4.15 ± 0.73^{e}	1.20 ± 0.41^{e}	5.00 ± 0.68^{cd}	4.00 ± 0.45^{e}	8.00 ± 2.06^{bc}	5.00 ± 0.66^{d}

Mean values with same letter in the same column are not significantly different at $p<0.05$ according to Duncan's Multiple Range Test (D.M.R.T) ± Standard Error.

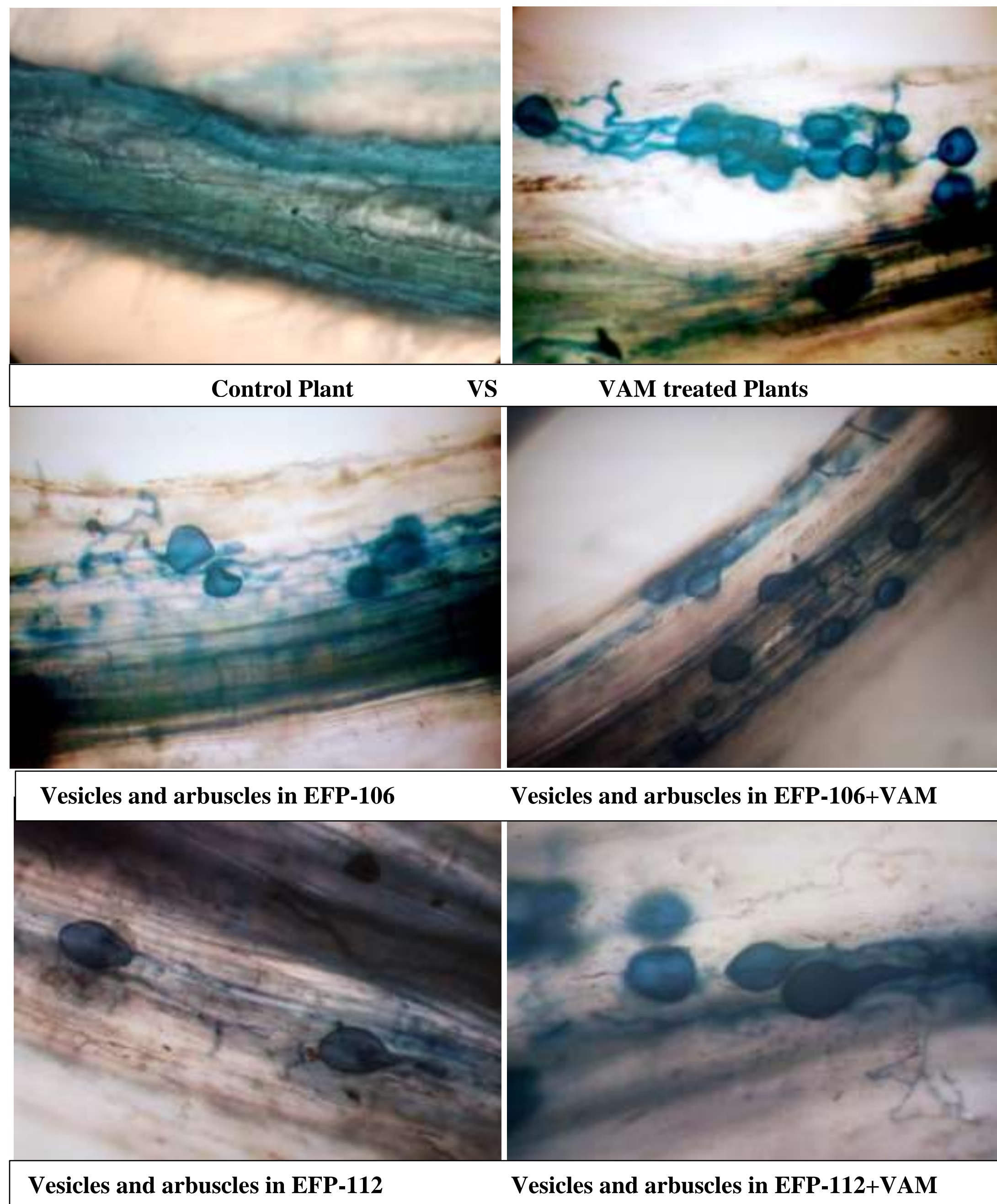

Fig. 1. Effect of endophytic fluorescence *Pseudomonas* and VAM fungi in the establishment and proliferation of VAM vesicles in roots of mung bean in sterilized soil**.**

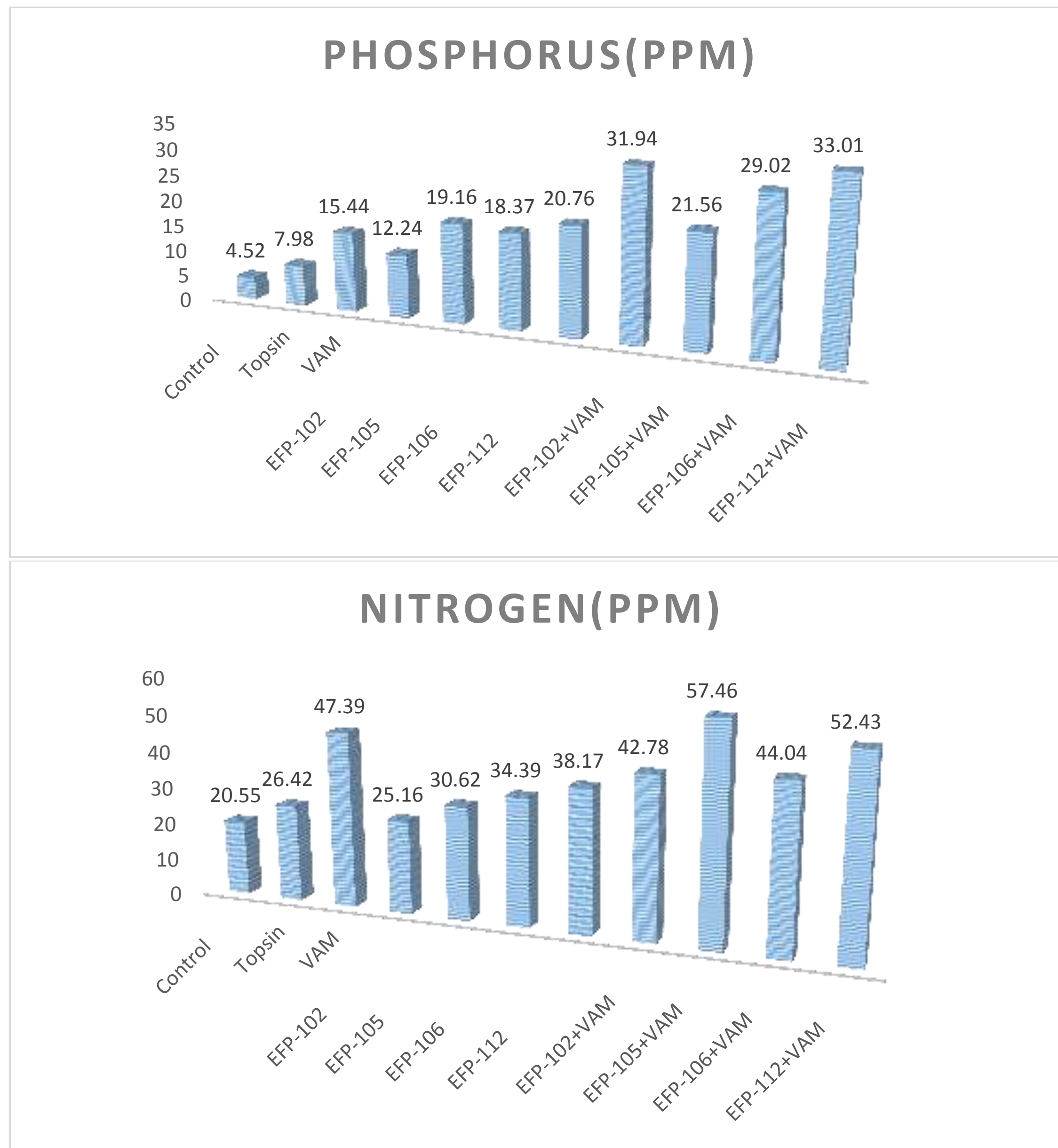

Fig. 2. Effect of EFP and VAM fungi on uptake of phosphorus (ppm) and nitrogen (ppm) in sterilized soil. Control = -ve control; Topsin = +ve control; Treatments = VAM, EFP-102, EFP-105, EFP-106, EFP-112, EFP-102+VAM, EFP-105+VAM, EFP-106+VAM, EFP-112+VAM.

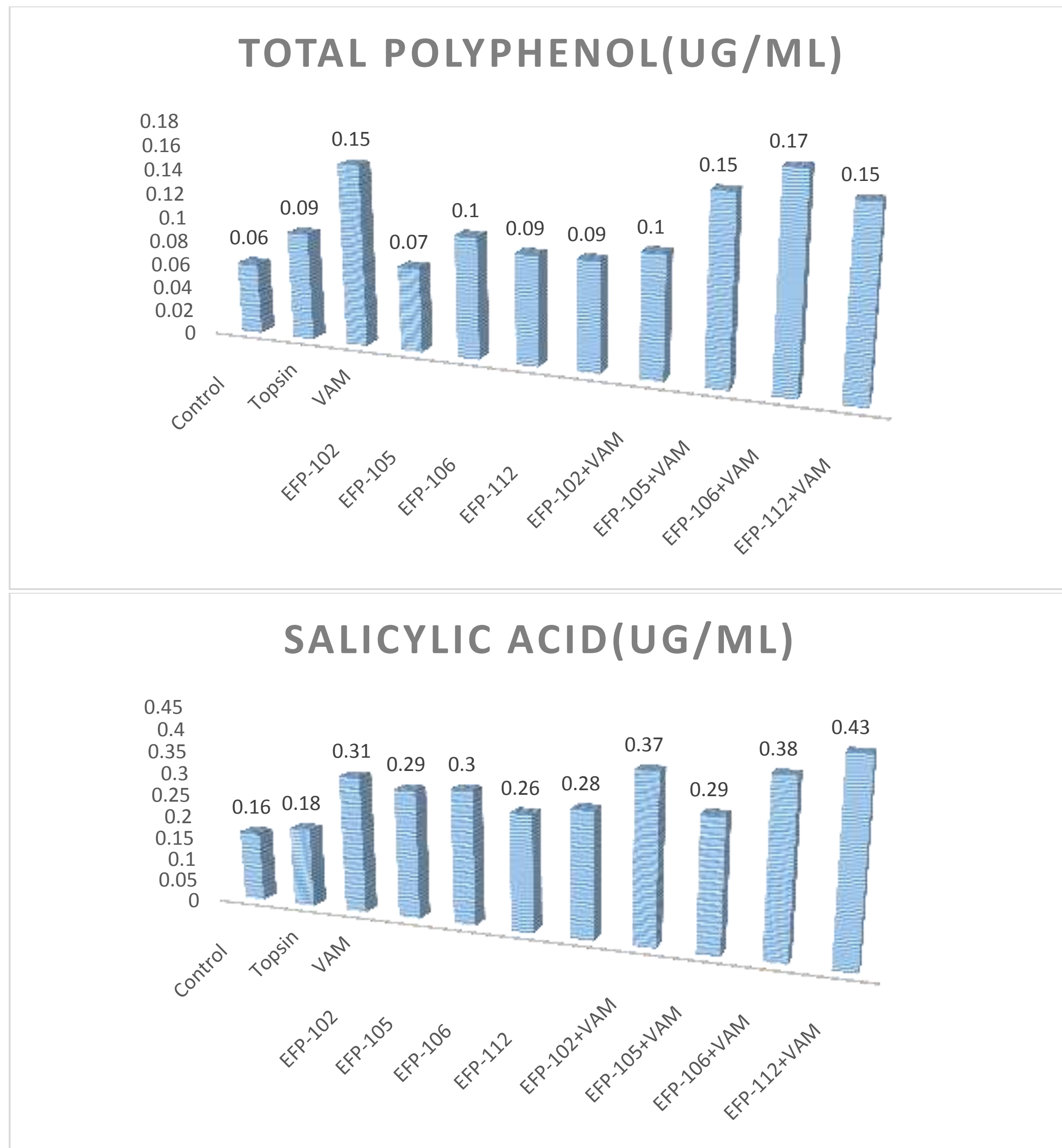

Fig. 3. Effect of EFP and VAM spore on concentration of total polyphenol (µg/mL) and salicylic acid (µg/mL) in sterilized soil. Control = -ve control; Topsin = +ve control; Treatments = VAM, EFP-102, EFP-105, EFP-106, EFP-112, EFP-102+VAM, EFP-105+VAM, EFP-106+VAM, EFP-112+VAM.

DISCUSSION

Bio-fertilizers are collections of naturally occurring elements that are used to improve soil fertility, improve plant growth, development and yield productivity (Sadhana, 2014). AMF have emphasized their uncountable benefits on soil health and crop efficiency. Therefore, it is broadly assumed that AMF could be considered as a replacement of inorganic fertilizers in the near future. Constant use of inorganic fertilizers, herbicides, and fungicides produced various problems to soil fertility, plants growth and human health and also damaged the quality of food products, and water systems (Yang *et al.,* 2004). Six isolates of endophytic fluorescent *Pseudomonas* tested against root rot fungi *viz*., *Rhizoctonia solani*, *Fusarium oxysporum, F. solani* and *Macrophomina phaseolina in vitro*. Prominent inhibition of root rot fungi observed by EFP isolates due to production of bioactive compounds as observed inhibition of several phytopathogenic fungi and bacteria *in vitro* for the control of *Septoria tritici* for wheat

leaf spot in USA. Kumar *et al.* (2015) also reported lysis of the fungal cells by endophytic *Pseudomonas* spp. Cell free culture filtrates of 04 screened isolates of EFP tested against root rotting fungi by disc diffusion method to check the biochemical activities of isolates. EFP-105 showed strong inhibition against *M. phaseolina* and *R. solani.* Zones of inhibition clearly indicated the potential effects of EFP isolates. Application of aqueous suspensions of bioactive isolates of EFP in screen house experiments showed improvement in plant growth of Mung bean whereas, filtrates were also used for the suppression of root infections by Korejo *et al.* (2017). Prominent shoot length and weight enhancement observed when EFP-102 and EFP-106 drenched the plants in sterilized soil by inducing systemic resistance against soil borne pathogens (Rahman *et al.,* 2017). Combined effects of VAM with EFP established the intra radical colonization of VAM effectively in the roots of mungbean. Present studies proved that use of VAM fungi in mungbean alone and combined with different isolates of EFP resulted in significant plant growth with greater masses by inducing systemic resistance (Shafique *et al.,* 2015). Research based on root infection by soil borne root rotting pathogens indicated that infection was reduced significantly by the application of VAM fungi alone and with active isolates of endophytic fluorescent *Pseudomonas* in sterilized soil because mycorrhizal fungi protect the plant from manifestation of soil borne pathogens as described by (Bokhari *et al.,* 2013) and suppressed root infection (Shafique *et al.,* 2015). Therefore, complete inhibition was shown in EFP-106+VAM against *R. solani* (in sterilized soil) however, significant inhibition can be seen in all treatments in mung beans.

Present study proved that by the application of VAM spores to mung beans resulting in high yield production with greater shoot and root lengths observed that in mycorrhizae significantly improved dry weight of stem and shoot. It is broadly assumed that VAM as biocontrol agents replace the inorganic fertilizers in coming days and effectiveness of mycorrhizal suspension can reduce the use of chemical fertilizers (Ortas, 2012). VAM fungi applied at large-scale fields in order to increase yield production of valuable crops such as maize (Sabia *et al.,* 2015). Likewise, PGPR improved yield productivity of staple crops i.e. wheat with high soluble phosphate fertilizer by producing antifungal compounds that is 2,4-diacetylphloroglucinol in order to suppress all diseases of wheat (Raajimakers and Weller, 1998). In our study, EFP isolates give promising results in repeated experiments on growth masses of test plants, were evaluated in pot experiments and repeated twice to confirm the results. In this study, VAM fungi applied with different isolates EFP as mycorrhizal helper bacteria (MHB) to the test plants to examine the combined effect and also assessed microscopically the intra radical colonization of VAM in form of vesicles as well as spores in soil which has been also described by Sumana *et al*. (2003). *Pseudomonas monteilii* has been reported for the stimulation of endomycorrhizal organization on the root of *Acacia holosericea* (Duponnois and Plenchette, 2003). Present studies are clearly revealed the establishment and proliferation of VAM fungi in the presence of EFP isolates resulting highest numbers of vesicles in roots of mungbean as Akhtar and Siddiqui, (2008) described on chickpea when *Glomus intraradices* applied with endophytic *Pseudomonas putida* and *Paenibacillus polymyxa*. Present research showed VAM-inoculated soil formed more constant masses of spores in mycorrhizospheric soil of mung beans than the non-VAM-inoculated soils.

In this research, it is clearly proved that AMF colonization enhanced root surface area stimulated the nutrients absorption from soil especially phosphorus (Riaz *et al.,* 2007).VAM spores inoculated with potential isolates of EFP enhanced phosphorus uptake by increasing root surface area in mung bean. The uptake of nitrogen also promoted with the application of VAM fungi with EFP isolates under sterilized soil as AMF have high ability to degrade polymeric N compounds in the function of amino acid absorption as compared to the Non-AMF plants (Näsholm, 2009). Mycorrhizal fungi not only protected plants from soil borne fungi (Bokhari *et al.,* 2022) by suppressed root infection but also induced systemic acquired resistance in plants via synthesis of salicylic acid which is natural phenolic compound against several nematodes, insect pests, bacterial and fungal infections. In present study, it is increased under the presence of VAM spores with EFP isolates (Hass and Defago, 2005). *Pseudomonas aeruginosa* also induced systemic resistance in plants by synthesis of salicylic acid. In this study, under screen house experiments, the total amount of phenolic contents were found significantly higher when VAM spores applied alone and with combined treatments of EFP isolates. Improvement of plant growth and reduced soil borne infections were apparently due to induction of systemic acquired resistance (SAR) as evident from production of higher concentration of phenolic compounds and salicylic acid in test plants. Plant health and crop productivity is directly linked to soil health. Poor arable land management and sterile soil often results in a negative feedback cycle in agricultural practices by an increase in soil borne pathogens and diseases. Use of chemical fertilizers such as adding lime, inorganic fertilizers, and pesticides can also change the physical and chemical nature of the soil environment. On contrary, soil biota also includes antagonistic species that are responsible for nutrient mineralization and cycling (biological control agents). Vesicular Arbuscular Mycorrhizae made symbiotic relations with approximately 90% of plant species (Ahanger *et al.,* 2014). VAM fungi inoculum is increasing due to their role in promotion of plant health, soil fertility, soil aggregates stability with increasing nutrient uptake and translocation especially nitrogen and phosphorus by significantly enhanced the access of roots to a large soil surface area (Bowles

et al., 2016). Currently, lots of attention has been devoted to selling mycorrhizal inoculants around the world for better growth of plants with quality. In this study, we found that VAM fungi inocula alone or in mix cultures with endophytic Fluorescent *Pseudomonas* strains gave promising advantage to encourage growth in order to supply professional horticulturists, greenhouse managers, nursery professionals and landscape professionals etc. across the country with this valuable technology but there is a weak traceability of the origin of the mycorrhizal fungi strains used in commercial. Continuous use of chemical fertilizers show negative impact on soil health and cause pollution which indirectly affect plant growth as well as toxic for human health, while bio fertilizers increase soil fertility with improving plant growth, yield production and reduce the chances of disease severity. Furthermore, laboratory based research on biocontrol to industry is a major aspect of our research studies, so the implementation of bio inoculants can be done easily. Vesicular Arbuscular mycorrhizal fungi (VAM) are frequently used as bio inoculants. Continuous use of chemical fertilizers show negative impact on soil health and cause pollution which indirectly affect plant growth as well as toxic for human health, while bio fertilizers increase soil fertility with improving plant growth, yield production and reduce the chances of disease severity.

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