IMPACT OF *GLOMUS FASCICULATUM* AND FLUORESCENT *PSEUDOMONAS* ON GROWTH PERFORMANCE OF *VIGNA RADIATA* (L.) WILCZEK CHALLENGED WITH PHYTOPATHOGENS

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Abstract: *Fusarium oxysporum* and *Rhizoctonia solani* are the major soil-borne pathogens causing growth and yield depression. The present study focused on the ability of fluorescent *Pseudomonas* and *Glomus fasciculatum* on growth performance of *Vigna radiata* in pathogen-infested soil. The percent colonization by *G. fasciculatum* indicated an increase of the presence of fluorescent *Pseudomonas* and a decrease of the presence of *Fusarium oxysporum* or *Rhizoctonia solani*. However, the reduction of colonization induced pathogen in percent was alleviated by fluorescent *Pseudomonas*. Inoculation with either fluorescent *Pseudomonas* or *G. fasciculatum* or both induced a significant increase in root and shoot length, plant vigour index, dry weight and total N and P content in *V. radiata* as compared to uninoculated control. The impact of inoculation was much pronounced in dual inoculated plants in comparison with those inoculated with either *G. fasciculatum* or fluorescent *Pseudomonas.* In contrast, treatment of plants with either *F. oxysporum* or *R. solani* decreased the root and shoot length, plant vigour index, dry weight and total N and P content in the test legume. However, in the presence of fluorescent *Pseudomonas* and *G. fasciculatum*, the adverse effect on the pathogens on growth of *V. radiata* was alleviated.

Key words: *Glomus fasciculatum*, fluorescent *Pseudomonas*, growth, *Vigna radiata*, phytopathogens

INTRODUCTION

The rhizosphere bacteria beneficial to plants are often referred to as Plant Growth Promoting Rhizobacteria or PGPR (Kloepper *et al.* 1989). The PGPR can positively influence plant growth by syntheizing plant growth promoting substances or by facilitating the uptake of certain nutrients from the environment. Among the PGPR, fluorescent pseudomonads received considerable interest because of their ability to suppress soil-borne plant pathogens, in addition to promoting plant growth.

Arbuscular mycorrhizal fungi (AM fungi) are of special interest in tropics because of their association with a large number of agricultural crop plants. The benefits of AM fungi include better uptake of nutrients, especially P, suppression of soil-borne plant pathogens, tolerance to water stress, production of plant growth hormones and mobilization of minor elements. The PGPR are synergistic with mycorrhizae in plant growth stimulation and may stimulate root colonization by mycorrhizal fungi (Bagyaraj and Menge 1978; Meyer and Linderman 1986; Chanway and Holl 1991). There are very few dual inoculation data of AM fungi and PGPR especially fluorescent *Pseudomonas* of crop plants. This study is aimed to recongnize the impact of dual inoculation involving fluorescent *Pseudomons* strain VuPf1 and *Glomus fasciculatum* on growth performance of *Vigna radiata* challenged with phytopathogens.

MATERIALS AND METHODS

Fluorescent *Pseudomonas*, *G. fasciculatum*, *F. oxysporum* and *R. solani* were obtained from the Department of Botany, Thiagarajar College, Madurai. Seeds of *V. radiata* variety VA02 were obtained from Tamil Nadu Agricultural Department, Madurai. Seeds of *V. radiata* were surface sterilized with 0.1% $HgCl_2$ for 2 min and bacterized with fluorescent *Pseudomonas* strain VuPf1. *F. oxysporum* and *R. solani* were cultivated in natural medium (sorghum seeds soaked in sucrose solution and autoclaved) and fungal cultures were incorporated into the sterile soil-sand mixture (2:1 ratio) in earthen pots. The following treatment schedule was followed:

T_1 Uninoculated control
T_2 Fluorescent *Pseudomonas* inoculation
T_3 *G. fasciculatum* inoculation
T_4 Fluorescent *Pseudomonas* + *G. fasciculatum* inoculation
T_5 *F. oxysporum* inoculation
T_6 *R. solani* inoculation
T_7 Fluorescent *Pseudomonas* + *F. oxysporum* inoculation
T_8 Fluorescent *Pseudomonas* + *R. solani* inoculation
T_9 *G. fasciculatum* + *F. oxysporum* inoculation

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T_{10} *G. fasciculatum* + *R. solani* inoculation
T_{11} Fluorescent *Pseudomonas* + *G. fasciculatum* + *F. oxysporum* inoculation
T_{12} Fluorescent *Pseudomonas* + *G. fasciculatum* + *R. solani* inoculation

Sterile tap water was used to water the plants. Plants were harvested 35 days after inoculation.

Plant materials were cut into bits and dried in an oven at 90°C for 3 days and dry weight was determined. The plant vigour index was determined by multiplying percent germination and root + shoot length. Fine roots were stained using trypan blue (Phillps and Hayman 1970) and the percent root colonization was calculated by grid-line intersect method (Giovannetti and Mosse 1980). Total nitrogen content was estimated according to the modified micro-Kjeldahl method (Umbriet *et al*. 1972). Acid-soluble total phosphorus was estimated by the method of Fiski-Subba Rao as modified by Bartlett (1959). The data were subjected to statistical analysis using IRRISTAT package for one way analysis of variance and Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Dual inoculation with fluorescent *Pseudomonas* and *G. fasciculatum* induced an increase in root and shoot length of *V. radiata* (Table 1). In contrast, the root and shoot length was significantly reduced following pathogen treatment. The pathogen-induced reduction in root and shoot length was compensated by mycorrhizal and fluorescent *Pseudomonas* inoculation. The data presented in figure 1. showed an increase in vigour index of *V. radiata* upon inoculation with either fluorescent *Pseudomonas* or *G. fasciculatum* or both. The index was drastically reduced by pathogen treatment. However, the pathogen-induced effect on the index was nullified upon fluorescent *Pseudomonas* + *G. fasciculatum* inoculation. Increased seed germination, root and shoot growth, greater seedling vigour index caused by *P. fluorescens* was demonstrated in several crops (Ramamoorthy *et al*. 2001; Khalid *et al*. 2004; Egamberdieva 2008). The increased plant growth reported here would possibly be the result of hormonal action since fluorescent *Pseudomonas* is able to produce substantial quantity of IAA and GA in culture medium (date not shown).

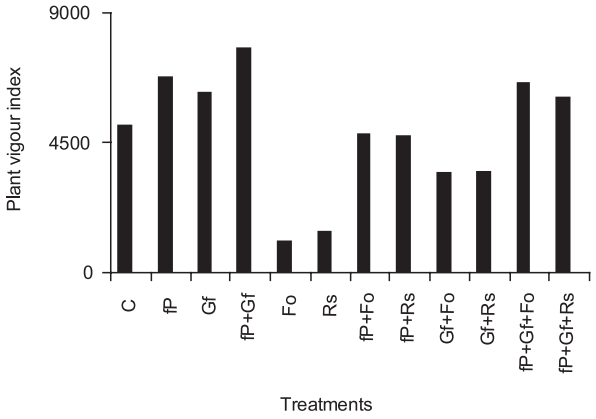

Fig. 1. Inoculation of fluorescent *Pseudomonas* and *G. fasciculatum* on vigour index of *V. radiata* grown in pathogen infested soil at 35 DAI

The results presented in table 2 showed that dual inoculation with fluorescent *Pseudomonas* and *G. fasciculatum* enhanced the mycorrhizal colonization. Earlier studies have also shown a positive influence of dual inoculation involving AM fungi and rhizobacteria on mycorrhizal colonization (Meyer and Linderman 1986; Duponnois and Plenchette 2003; Gamalero 2004; Akhtar and Siddique 2008). Reduction in percent colonization of *G. fasciculatum* in the presence of *F. oxysporum* or *R. solani* and alleviation of pathogen effect by colonization with mycorrhizal fungi and fluorescent *Pseudomonas* could be due to the produc-

Table 1. Inoculation of fluorescent *Pseudomonas* and *G. fasciculatum* on root and shoot length of *V. radiata* grown in pathogen infested soil at 35 DAI

Treatment	Root length [cm/plant]	Shoot length [cm/plant]
Uninoculated control	30.66 d ± 2.08	20.00 b ± 4.58
Fluorescent *Pseudomonas*	32.66 de ± 1.71	35.00 g ± 2.00
Glomus fasciculatum	31.33 d ± 1.15	30.66 de ± 1.73
Fluorescent *Pseudomonas* + *G. fasciculatum*	36.66 e ± 2.93	40.83 g ± 2.75
Fusarium oxysporum	13.83 a ± 1.75	7.5 a ± 1.32
Rhizoctonia solani	17.33 ab ± 0.57	8.0 a ± 1.46
Fluorescent *Pseudomonas* + *F. oxysporum*	24.00 c ± 0.58	23.66 c ± 1.08
Fluorescent *Pseudomonas* + *R. solani*	24.16 c ± 2.75	23.00 c ± 3.46
G. fasciculatum + *F. oxysporum*	18.83 b ± 0.78	28.66d e ± 2.08
G. fasciculatum + *R. solani*	18.50 b ± 2.18	27.16 d ± 0.43
Fluorescent *Pseudomonas* + *G. fasciculatum* + *F. oxysporum*	31.00 d ± 0.57	34.33 f ± 0.57
Fluorescent *Pseudomonas* + *G. Fasciculatum* + *R. Solani*	29.00 d ± 1.26	31.50 ef ± 0.59
F value	34.35	88.53

± Standard deviation
$p < 0.05$ values marked with different letters in the same column indicates significant differences

Table 2. Influence of inoculation of fluorescent *Pseudomonas* and *G. fasciculatum* on mycorrhizal infection in roots of *V. radiata* grown in pathogen infested soil at 35 DAI

Treatment	Hyphal infection	Vesicular infection
Glomus fasciculatum	49.39 cd ± 2.40	28.69 b ± 3.02
Fluorescent *Pseudomonas* + *G. fasciculatum*	51.19 d ± 2.06	35.13 c ± 1.59
G. fasciculatum + *F. oxysporum*	38.66 b ± 2.30	21.33 a ± 2.30
G. fasciculatum + *R. solani*	35.23 a ± 1.65	18.28 a ± 1.67
Fluorescent *Pseudomonas* + *G. fasciculatum* + *F. oxysporum*	44.00 b ± 4.00	26.66 b ± 2.30
Fluorescent *Pseudomonas* + *G. fasciculatum* + *R. Solani*	45.00 bc±1.00	26.64 b ± 1.51
F value	13.90	22.58

± Standard deviation
p < 0.05 values marked with different letters in the same column indicates significant differences

tion of antifungal compound. However, it is worthwhile to note that antifungal compounds produced by *Pseudomonas* spp did not interfere with AM formation or functioning (Barea *et al*. 1998; Vazquez *et al*. 2000).

The PGPR are synergistic with mycorrhizae in stimulating plant growth (Meyer and Linderman 1986; Chanway and Holl 1991). As shown in table 3 and 4 dual inoculation with fluorescent *Pseudomonas* and *G. fasciculatum* enhanced the plant biomass, N and P accumulation as compared to single inoculation with either fluorescent *Pseudomonas* or *G. fasciculatum*. In an earlier report, Akhtar and Siddique (2008) observed a synergistic interaction between *G. intraradices* and *P. alcaligens* which resulted in increased biomass, total nitrogen and total phosphorus accumulation. The ill-effects of *F. oxysporum* and *R. solani* on plant biomass, and N and P accumulation were significantly reduced upon fluorescent *Pseudomonas* + *G. fascicultum* inoculation.

It is clearly noted that fluorescent *Pseudomonas* synergistic with mycorrhiza enhances the plant growth and biomass by producing plant growth promoting substances on one hand and antibiotics on the other hand, but without affecting the formation and functioning of mycorrhizal fungi. The present study revealed that an efficient biocontrol strain of fluorescent *Pseudomonas* strain VuPf1 in combination with *G. fasciculatum* alleviated the adverse effects of the pathogens in *V. radiata*. Thus fluorescent *Pseudomonas* and *G. fasciculatum* can be exploited for enhancing the productivity of *V. radiata* even if the soils are infested with phytopathogens.

Table 3. Influence of inoculation of fluorescent *Pseudomonas* and *G. fasciculatum* on dry weight accumulation in *V. radiata* grown in pathogen infested soil at 35 DAI

Treatment	Root Dry wt. [g/plant]	Shoot Dry wt. [g/plant]
Uninoculated control	0.19 bcd ± 0.04	0.47 bcd ± 0.06
Fluorescent *Pseudomonas*	0.31 ef ± 0.02	0.99 f ± 0.19
Glomus fasciculatum	0.29 e ± 0.05	0.86 ef ± 0.12
Fluorescent *Pseudomonas* + *G. fasciculatum*	0.32 ef ± 0.09	1.45 g ± 0.14
Fusarium oxysporum	0.02 a ± 0.004	0.11 a ± 0.03
Rhizoctonia solani	0.05 ab ± 0.03	0.24 ab ± 0.08
Fluorescent *Pseudomonas* + *F. oxysporum*	0.22 d ± 0.05	0.737 def ± 0.05
Fluorescent *Pseudomonas* + *R. solani*	0.21 cd ± 0.06	0.57 cde ± 0.04
G. fasciculatum + *F. oxysporum*	0.09 abc ± 0.01	0.31 abc ± 0.02
G. fasciculatum + *R. solani*	0.10 abc ± 0.01	0.30 abc ± 0.07
Fluorescent *Pseudomonas* + *G. fasciculatum* + *F. oxysporum*	0.29 d ± 0.05	0.75 def ± 0.10
Fluorescent *Pseudomonas* + *G. fasciculatum* + *R. Solani*	0.24 d ± 0.08	0.81 def ± 0.09
F value	3.98	12.61

± Standard deviation. p < 0.05 values marked with different letters in the same column indicates significant differences

Table 4. Influence of inoculation of fluorescent *Pseudomonas* and *G. fasciculatum* on total nitrogen content and total phosphorus content of *V. radiata* grown in pathogen infested soil at 35 DAI

Treatment	Total nitrogen content [mg N/plant]		Total phosphorus content [mg P/plant]	
	root	shoot	root	shoot
Uninoculated control	4.56 ab ± 0.93	17.50 bcd ± 0.12	0.39 abc ± 0.08	0.77 abc ± 0.08
Fluorescent *Pseudomonas*	16.61 d ± 0.7	52.52 fg ± 3.48	0.92 de ± 0.08	2.884 f ± 0.10
Glomus fasciculatum	13.12 cd ± 0.50	49.01 f ± 2.20	0.72 cde ± 0.05	2.29 ef ± 0.05
Fluorescent *Pseudomonas* + *G. fasciculatum*	17.21 d ± 1.27	79.18 g ± 4.51	1.09 e ± 0.05	6.26 g ± 1.58
Fusarium oxysporum	0.07 a ± 0.02	1.51 a ± 0.36	0.014 a ± 0.004	0.08 a ± 0.004
Rhizoctonia solani	0.98 ab ± 0.09	4.07 ab ± 0.30	0.05 a–d ± 0.01	0.238 ab ± 0.05
Fluorescent *Pseudomonas* + *F. oxysporum*	7.46 abc ± 1.04	29.11 de ± 0.65	0.67 cde ± 0.08	1.87 de ± 0.18
Fluorescent *Pseudomonas* + *R. solani*	5.96 abc ± 0.75	20.41 cde ± 0.48	0.52 bcd ± 0.15	1.10 bcd ± 0.25
G. fasciculatum + *F. oxysporum*	2.08 ab ± 0.50	8.08 bcd ± 0.65	0.13 ab ± 0.03	0.43 ab ± 0.05
G. fasciculatum + *R. solani*	2.24 ab ± 0.08	5.8 abc ± 0.82	0.18 ab ± 0.07	0.455 ab ± 0.07
Fluorescent *Pseudomonas* + *G. fasciculatum* + *F. oxysporum*	8.00 abc ± 1.58	41.41 de ± 3.01	0.69 cde ± 0.05	1.835 de ± 0.13
Fluorescent *Pseudomonas* + *G. fasciculatum* + *R. solani*	8.65 bc±0.32	38.60 e± 1.10	0.81 cde ± 0.08	1.49 cd ± 0.66
F value	5.98	17.14	4.73	38.82

± Standard deviation
$p < 0.05$ values marked with different letters in the same column indicates significant differences

REFERENCES

Akhtar M.S., Siddique Z.A. 2008. *Glomus intraradices*, *Pseudomonas alcaligens*, and *Bacillus pumilus*: effective agents for the control of root – rot disease complex of chick pea (*Cicer arietinum* L.). J. Gen. Plant Pathol. 74, p. 53.

Bagyaraj D.J., Menge J.A. 1978. Interaction between VA mycorrhizal fungus and *Azotobacter* and their effects on rhizosphere microflora and plant growth. New Phytol. 80, p. 567.

Barea J.M., Andrade G., Biancioto V., Dowling D., Lohrke S., Bonfante P., O'Gara F., Azcon-Aguilar. 1998. Impact on Arbuscular Mycorrhiza formation of *Pseudomonas* strains used as inoculants for biocontrol of soil-borne fungal plant pathogens. Appl. Environ. Microbiol. 64, p. 2304.

Bartlett G.R. 1959. Phosphorus assay in column chromatography. J. Biol. Chem. 234, p. 446.

Chanway C.P., Holl F.B. 1991. Biomass increase and associative nitrogen fixation of mycorrhizal *Pinus contorta* seedlings inoculated with a plant growth promoting *Bacillus* strain. Can. J. Bot. 69, p. 507.

Duponnois R., Plenchette C.A. 2003. Mycorrhiza helper bacterium enhances ectomycorrhizal and endomycorrhizal symbiosis of Australian *Acacia* species. Mycorrhiza 13, p. 85.

Egamberdieva D. 2008. Plant growth promoting properties of Rhizobacteria isolated from wheat and pea grown in loamy sand soil. Turk. J. Biol. 32, p. 9.

Gamalero E., Trotta A., Massa N., Copetta A., Martinotti M.G., Berta G. 2004. Impact of two fluorescent pseudomonads and an arbuscular mycorrhizal fungus on tomato plant growth, root architecture and P acquisition. Mycorrhiza 14, p. 185.

Giovannetti M., Mosse B. 1980. An evaluation of techniques for measuring vesicular-arbuscular infections in roots. New Phytol. 84, p. 489.

Khalid A., Arshad M., Zahir Z.A. 2004. Screening plant growth – promoting rhizobacteria for improving growth and yield of wheat. J. Appl. Microbiol. 96: 473–480.

Kloepper J.W., Lifshitz R., Zablotowics R.M. 1989. Free-living bacterial inocula for enhancing crop productivity. Trends Biotechnol. 7, p. 39.

Meyer J.R., Linderman R.G. 1986. Response of substerranean clover to dual inoculation with vesicular-arbuscular mycorrhizal fungi and a plant growth-promoting bacterium, *Pseudomonas putida*. Soil Biol. Biochem. 18, p. 185.

Phillps J.M, Hayman D.S. 1970. Improved procedures for clearing and staining parasitic and vesicular- arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55, p. 158.

Ramamoorthy V., Viswanathan R., Raguchander T., Prakasam V., Samiyappan R. 2001. Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. Crop Protect. 20, 1–11.

Umbriet W.W, Burris R.H., Stauffer J.F. 1972. Methods for nitrogen, in Manometric and biochemical techniques. 5th ed. Burgess Publishing Company, Minnesota, 259 pp.

Vazquez M.M, Cesar S.A, Azcon R., Barea J.M. 2000. Interaction between mycorrhizal fungi and other microbial inoculants (*Azospirillum, Pseudomonas, Trichoderma*) and their effects on microbial population. Appl. Soil Ecol. 15, p. 261.

POLISH SUMMARY

DZIAŁANIE *GLOMUS FASCICULATUM* I FLUORYZUJĄCEGO *PSEUDOMONAS* NA WZROST *VIGNA* RADIATA (L.) WILCZEK PORAŻONĄ PRZEZ PATOGENY

Fusarium oxysporum i *Rhizoctonia solani* są głównymi patogenami przenoszącymi się poprzez glebę, ograniczającymi wzrost i plon roślin. Badano wpływ fluoryzującego *Pseudomonas* i *Glomus fasciculatum* na wzrost *V. radiata* w glebie zakażonej patogenami. Procent zasiedlenia przez

G. fasciculatum powodował wzrost obecności fluoryzującego *Pseudomonas* i spadek występowania *F. oxysporum* lub *R. solani*. Jednak indukowana przez patogena redukcja procentu zasiedlenia była ograniczona przez fluoryzujący *Pseudomonas*. Inokulacja fluoryzującym *Pseudomonas* lub *G. fasciculatum* lub obydwoma mikroorganizmami, indukowała znaczny wzrost długości korzeni i pędów, wskaźnika wigoru roślin, zawartości suchej masy i zawartości ogólnego N i P u *V. radiata,* w porównaniu do kontroli. Wpływ inokulacji był o wiele wyraźniejszy w przypadku podwójnej inokulacji roślin, w porównaniu do roślin inokulowanych *G. fasciculatum* lub fluoryzującym *Pseudomonas*. Przeciwnie, potraktowanie roślin *F. oxysporum* lub *R. solani* powodowało spadek długości korzeni i pędów, wskaźnika wigoru roślin, suchej masy i zawartości ogólnego N oraz P w testowanej roślinie motylkowej. Jednakże w obecności fluoryzującego *Pseudomonas* i *G. fasciculatum* niekorzystne działanie na wzrost patogenów było złagodzone.