Int. J. Biol. Res., 6(1): 27-33, 2018.

IMPACT OF ENDO-NODULE FLUORESCENT *PSEUDOMONAS* AND RHIZOBIA ON ROOT ROTTING FUNGI AND GROWTH OF SOYBEAN (*GLYCINE MAX* L. MERR)

Rubina Noreen[1], Habiba[2], Faizah Urooj[1], Hafiza Farhat[1], Hafiza Asma Shafique[1], Afshan Rahman[1] and Syed Ehteshamul-Haque[1*]

[1]*Agricultural Biotechnology & Phytopathology Laboratory, Department of Botany, University of Karachi, Karachi 75270, Pakistan*
[2]*Postharvest & Food Biochemistry Laboratory, Department of Food Science & Technology, University of Karachi, Karachi 75270, Pakistan*
[*]*Corresponding author's email: sehaq@uok.edu.pk*

ABSTRACT

Rhizobia and fluorescent *Pseudomonas* associated with root nodules of mungbean (*Vigna radiata*) were studied for their synergistic effect on disease suppression and promotion of plant growth. Fluorescent *Pseudomonas* viz., NAFP-19, NAFP-31 and NAFP-32 and *Rhizobium vignae* (NFB-103), *R. vignae* (NFB-107) and *R. vignae* (NFB-109) were applied simultaneously in naturally infested soil in screen house experiment caused suppression of root rotting fungi of soybean as compared to untreated control. Combined application of both bacteria produced healthier plants than untreated plants or plants inoculated with either component alone.

KEYWORDS: Rhizobia, Fluorescent *Pseudomonas*, Biocontrol, Root rotting fungi, Soybean.

INTRODUCTION

Soybean (*Glycine max* L. Merr) is important leguminous crop which is a source of food for man and livestock (Dwevedi and Kayastha, 2011). Soybean consists of high quality protein ranging from 37 to 42% (Wilcox and Shibles, 2001), ash is 6%, carbohydrate is 29% and oil ranges from 17-24% (Ali *et al.*, 2015) which makes it an important crop. Seeds are highly proteinaceous because of assimilation of high concentration of nitrogen in the shoot of plant therefore it requires high amount of nitrogen (Board, 2013). Soybean can be grown in different environmental conditions because of its adaptations (Tran *et al.*, 2015). Nitrogen is either provided through biological nitrogen fixation by specific rhizobia (Seneviratne *et al.*, 2000) or by dissolving mineral nitrogen from soil (Albareda *et al.*, 2009). Growers use BNF as biofertilizer for sustainable agricultural practices (Janagard and Ebabi- Segherloo, 2016). In order to sustain agricultural system, leguminous plants are inoculated with rhizobia (Howieson *et al.*, 2007).

Soybean is known to inhabit a wide range of microorganism i.e., *Bradyrhizobium* species (Zhang *et al*., 2012), *Rhizobium Sinorhizobium* species (Saldana *et al*., 2003) and *Agrobacterium* species (Youseif *et al*., 2014) which are responsible of fixing nitrogen and increasing yield of crop. Plant growth promoting rhizobacteria (PGPR) are associated with root nodules other than rhizobia (Martinez-Hedalgo and Hirsch, 2017). PGPR which are known to be associated with soybean i.e., *Pseudomonas, Bacillus, Enterobacter* and

Microbacterium which are able to fix nitrogen (Park *et al.*, 2005), sequester iron (Susilowatl *et al.*, 2011) phytohormones producer (Masciarelli *et al.*, 2014), inorganic phosphate solubilizer (Sharma *et al*, 2012), and fungal or viral pathogens suppresser (Khalimi and Suprata, 2011). Suppression of root rotting fungi by rhizobia (Ehteshamul-Haque and Ghaffar 1993) along with endo-nodule fluorescent *Pseudomonas* (Farhat *et al*., 2017). The effect of indigenous rhizobial effect can be overcome by using specific PGPR with rhizobia (Aung *et al*., 2013)**.**

Wide range of crops are found to be extensively associated with fluorescent *Pseudomonas* which induces systemic resistance in plants (Shafique *et al.*, 2015) and also known to have suppressive potential against root infecting fungi (Siddiqui and Ehteshamul-Haque 2001). Antifungal metabolites are also produced by fluorescent *Pseudomonas* which reduces associated fungal pathogens in soil (Rahman *et al.*, 2016). Root nodule associated with Fluorescent *Pseudomonas* is for its biocontrol ability (Noreen *et al.,* 2015). In our previous study we have reported the biocontrol potential of mungbean's nodules linked fluorescent *Pseudomonas* and rhizobia against root infecting fungi and root knot nematode (Noreen *et al.*, 2015). It is reported that number of nodules in chickpea was greater in those plants treated with both rhizobia and fluorescent *Pseudomonas* than rhizobia alone (Noreen *et al.*, 2016). The role of root nodule associated fluorescent *Pseudomonas* also reported in nodule formation (Noreen *et al.*, 2019). The present report describes the effect of fluorescent *Pseudomonas* and rhizobia on growth of soybean. Co-inoculation of rhizobia with proper plant growth promoting rhizobacteria (PGPR) is one of the popular approaches to overcome the effect of indigenous rhizobia (Aung *et al*., 2013)**.**

MATERIALS AND METHODS

Bacterial cultures of fluorescent *Pseudomonas* and Rhizobia: Cultures of fluorescent *Pseudomonas* and rhizobia used in present study were isolated from nodules of mungbean (*Vigna radiata* L.) by Noreen *et al*., 2015; 2019).

Role of *Pseudomonas* and rhizobia in screen house experiment: Soil was collected from Department of Botany, University of Karachi having 8.0 pH and 40% moisture holding capacity un-sterilized sandy loam was transferred into earthen pots of 12 cm diameter with 1 kg of soil per pot. Bacterial sample (25 mL) of NAFP-19 (3.3×10^8 cfu/mL), NAFP-32 (2.5×10^8 cfu/mL), NAFP-31 (1.4×10^8 cfu/mL), *Rhizobium vignae* (NFB-103) (3.6×10^8 cfu/mL), *R. vignae* (NFB-107) (3.4×10^8 cfu/mL) and *R. vignae* (NFB-109) (2.2×10^8 cfu/mL) was added after sowing six soybean (*Glycine max* L. Merr) seeds in each pot. Untreated plants functioned as negative control, while for positive control 25 mL of carbendazim (200 ppm) per pot was used. Complete randomized block design was used for experiment with four replicates; four seedlings were kept in each pot. After 45 days, plants were uprooted and root shoot biomass was documented. Infection percentage of root rotting fungi was calculated as described by Urooj *et al.,* (2018).

Analysis of data: For plant growth parameters, one way ANOVA was used while Two-way ANOVA was used to compare the means of infection of fungal pathogens and among the treatments. Least significant difference (LSD) test at ($p = 0.05$) was calculated to compare the means as the follow up of ANOVA (Gomez and Gomez, 1984).

RESULTS

In the screen house experiment, some effective isolates of *Pseudomonas* viz., NAFP-19, NAFP-32, NAFP-31 and rhizobia viz., *Rhizobium vignae* (NFB-103), *R. vignae* (NFB-107) and *R. vignae* (NFB-109) were used as biocontrol agent against root infecting fungi individually or in combination. In the present study, soybean plant showed greater height treated with *R. vignae* (NFB-107) alone or co-inoculated with NAFP-31 or NAFP-32 while maximum weight of shoot was observed in treatment of NAFP-31 + *R. vignae* (NFB-107). Maximum root length was observed in plants treated with NAFP-19 + *R. vignae* (NFB-107) and *R. vignae* (NFB-103) while maximum root weight was observed in NAFP-31 + *R. vignae* (NFB-107) and NAFP-103 as compared to control (Table 1). Maximum suppression of *M. phaseolina* was observed in plants treated with NAFP-19 + *R. vignae* (NFB-103) and NAFP-32 + *R. vignae* (NFB-109) in comparison to control. Significant suppression of *R. solani* was observed by combined treatment of NAFP-19 + *R. vignae* (NFB-103), NAFP-32 + *R. vignae* (NFB-109) in comparison to control. Maximum inhibition of *F. solani* was observed in positive control (carbendazim) while suppression of *F. oxysporum* was observed in individual treatment of *R. vignae* (NFB-103) (Table 2).

Table 1. Effect of soil drench with different isolates of *Pseudomonas* and rhizobia on growth of soybean plants in screen house experiment.

Treatments	Growth parameter			
	Shoot length (cm)	**Fresh shoot weight (g)**	**Root length (cm)**	**Fresh root weight (g)**
Control	27.06	1.02	16.60	0.34
Carbendazim	31.50	1.71	18.29	0.53
NAFP-19	39.12	2.53	24.56	0.59
NAFP-32	36.93	2.17	23.18	0.54
NAFP -31	40.31	1.88	19.25	0.56
R. vignae (NFB-107)	45.43	2.49	21.68	0.54
R. vignae (NFB-109)	39.65	1.63	21	0.34
R. vignae (NFB-103)	41.03	1.59	25.18	0.42
NAFP-19 + *R. vignae* (NFB-107)	44.18	1.64	25.59	0.39
NAFP-19 + *R. vignae* (NFB-109)	42.62	1.86	19.5	0.51
NAFP-19 + *R. vignae* (NFB-103)	40.68	2.05	22.43	0.46
NAFP-32 + *R. vignae* (NFB-107)	43.08	2.28	20.72	0.52
NAFP-32 + *R. vignae* (NFB-109)	35.62	2.07	22.68	0.58
NAFP-32 + *R. vignae* (NFB-103)	35.78	2.46	22.10	0.54
NAFP-31 + *R. vignae* (NFB-107)	34.65	3.07	23.52	0.74
NAFP-31 + *R. vignae* (NFB-109)	35.56	1.98	19.09	0.57
NAFP-31 + *R. vignae* (NFB-103)	30.18	1.97	16.65	0.45
$LSD_{0.05}$	**7.96**[1]	**1.23**[1]	**7.70**[1]	**0.30**[1]

[1]Mean values in column showing differences greater than LSD values are significantly different at $p<0.05$

Table 2. Effect of different isolates of *Pseudomonas* and rhizobia on root infection by *Macrophomina phaseolina, Rhizoctonia solani Fusarium solani* and *F. oxysporum* on mungbean roots in screen house experiment.

Treatments	**Infection %**			
	M. phaseolina	***R. solani***	***F. solani***	***F. oxysporum***
Control	75	68.75	62.5	37.5
Carbendazim	56.25	75	25	37.5
NAFP-19	93.75	75	43.75	37.5
NAFP-32	75	56.25	43.75	43.75
NAFP -31	81.25	87.5	37.5	31.25
R. vignae (NFB-107)	87.25	56.25	93.75	56.25
R. vignae (NFB-109)	81.25	81.25	87.5	37.5
R. vignae (NFB-103)	81.25	56.25	87.5	25
NAFP-19 + *R. vignae* (NFB-107)	50	56.25	81.25	50
NAFP-19 + *R. vignae* (NFB-109)	56.25	43.75	68.75	68.75
NAFP-19 + *R. vignae* (NFB-103)	37.5	81.25	93.75	50
NAFP-32 + *R. vignae* (NFB-107)	75	62.5	68.75	56.25
NAFP-32 + *R. vignae* (NFB-109)	56.25	31.25	56.25	56.25
NAFP-32 + *R. vignae* (NFB-103)	37.5	31.25	93.75	68.75
NAFP-31 + *R. vignae* (NFB-107)	62.5	37.5	75	31.25
NAFP-31 + *R. vignae* (NFB-109)	93.75	43.75	62.5	62.5
NAFP-31 + *R. vignae* (NFB-103)	68.75	68.75	93.75	31.25
$LSD_{0.05}$	**Treatments = 43.34[1]**		**Pathogens = 21.02[2]**	

[1]Mean values in column showing differences greater than LSD values are significantly different at $p<0.05$
[2]Mean values in rows showing differences greater than LSD values are significantly different

DISCUSSION

Symbiotic relationship between rhizobia and legumes vigorously fix nitrogen besides it is beneficial to production of crop (Pepper, 2000). Crop production can be increased by plant growth promoting bacteria (PGPB) besides rhizobia combined treatment. Most of the strains of PGPB have been tested along with species of common rhizobacteria such as *Bradyrhizobium* or *Rhizobium* (Bullied *et al.*, 2002). Egamberdiyeva *et al.* (2010) reported that combined treatment of fodder galega with root colonizing species of *Pseudomonas* and *Rhizobium* enhanced growth and symbiotic performance of host. In the current study, three isolates each of fluorescent *Pseudomonas* and rhizobia isolated from root nodules of *Vigna radiata* were inoculated simultaneously to check their enhancement on the growth of soybean.

In this study, increased biomass of shoot and root of soyabean was observed in combined treatment of fluorescent *Pseudomonas* and rhizobia which was similar to the results reported by Egamberdiyeva *et al.* (2010). Significant increase in plant height and shoot biomass was observed in plants inoculated with *Rhizobium* sp. in soybean seeds than un-inoculated plants (Janagard and Ebadi-Segherloo, 2016). The increase in yield of soybean plants was observed by inoculation of *Rhizobium* without any addition of chemical N fertilizer even when bradyrhizobial population of soybean is already present in soil (Hungria *et al*., 2015). The biologically active compounds such as phytohormones i.e., auxins, siderophores as well as different enzymes are reported to be produced by bacteria (Oswald *et al.,* 2010).

Inoculation of isolates of *Bradyrhizobium* has been reported to increase growth and yield of soybean seeds (Kala *et al.*, 2011). Salih *et al.*, 2015, reported increase in yield and relative growth rate of soybean by inoculation of BNF. Application of *Bradyrhizobium japonicum* has been reported to increase plant height and shoot biomass of soybean (Zuffo *et al.*, 2015). Higher number of pods, nodule dry weight, seed number and grain yield has been observed in inoculated plants (Mohamed and Hassan, 2015). Ntambo *et al.* (2017) studied the use *Bradyrhizobium japonicum* under field conditions in order to reduce the use of nitrogen fertilizers for soybean. The present study revealed the role of fluorescent *Pseudomonas* associated with root nodules by increasing shoot and root mass and suppressing the infection of root infecting fungi.

CONCLUSION

Pseudomonas and rhizobia isolates, as used in this study, have potential to increase plant growth of *G. max*. Their co-inoculation could be a valuable crop-management-tool against soil borne root infecting fungi and parasitic nematodes.

ACKNOWLEDGEMENTS

Financial assistance provided by the Dean, Faculty of Science, University of Karachi, is sincerely acknowledged.

REFERENCES

Albareda, M., D.N. Rodríguez-Navarro and F.J. Temprano. (2009). Soybean inoculation: Dose, N fertilizer supplementation and rhizobia persistence in soil. *Field Crops Research*, 113(4): 352-356.

Ali, A., S.A. Khan, E. Khan, N. Ali, I. Hussain and F. Ahmad. (2015). Genetic studies among diverse soybean (*Glycine max* L. Merrill) genotypes for variability and correlation at Swat. *International Journal of Biosciences*, 6(4): 165-169.

Aung, T.T., B. Buranabanyat, P. Piromyou, A. Longtonglang, P. Tittabutr, N. Boonkerd and N. Teaumroong. (2013). Enhanced soybean biomass by co-inoculation of *Bradyrhizobium japonicum* and plant growth promoting rhizobacteria and its effects on microbial community structures. *African J. Microbiol. Res*., **7**: 3858-3873.

Board, J.E. (2013). A Comprehensive survey of international soybean research-genetics, physiology, Agronomy and Nitrogen Relationships. *Ana. Pantar. Croatia*, 1-624 p.

Bullied, J., T.J. Buss and J.K. Vessey. (2002). *Bacillus cereus* UW85 inoculation effects on growth, nodulation, and N accumulation in grain legumes: Field studies. *Can. J. Plant Sci*., 82: 291-298.

Dwevedi, A. and A.M. Kayastha. (2011). Soybean: a multifaceted legume with enormous economic capabilities. In: Tzi-Bun N editor. Soybean –Biochemistry, Chemistry and Physiology. In Tech Europe University Campus STeP Ri Slavka Krautzeka 83/A 51000 Rijeka, Croatia.

Egamberdiyeva, D., G. Berg, K. Lindström and L.A. Räsänen. (2010). Co-inoculation of *Pseudomonas* spp. with Rhizobium improves growth and symbiotic performance of fodder galega (*Galega orientalis* Lam.). *Eur. J. Soil Biol*., 46: 269-272.

Ehteshamul-Haque, S. and A. Ghaffar. (1993). Use of rhizobia in the control of root rot diseases of sunflower, okra, soybean and mungbean. *J. Phytopathol*., 138: 157-163.

Farhat, H., F. Urooj, H.A. Shafique, V. Sultana and S. Ehteshamul-Haque. (2017). Rhizobia suppress the root knot nematode and fungi on mungbean. *Int. J. Biol. Res*., 5(2): 71-75.

Gomez, K.A. and A.A. Gomez. (1984). *Statistical Procedures for Agricultural Research*. 2nd ed. Wiley, New York. Pp. 680.

Howieson, J.G., R.J. Yates, K.J. Foster, D. Real and R.B. Besier. (2007). In: Dilworth, M.J., James, E.K., Sprent, J.I. & Newton, W.E. (Eds.) *Prospects for the future use of legumes*. In nitrogen-fixing leguminous symbioses. Springer, Netherlands. 363-394.

Hungria, M., M.A. Nogueira and R.S. Araujo. (2015). Soybean seed co-inoculated with *Bradyrhizobium* spp. and *Azospirillum brasilense*. A new biotechnological tool improve yield and sustainability. *American Journal of Plant Science*, 06(06): 811-817.

Janagard, M.S. and A. Ebadi-Segherloo. (2016). Inoculated soybean response to starter nitrogen in conventional cropping system in Moghan. *Journal of Agronomy*, 15(1): 26-32.

Kala, T.C., R.M. Christi and N.R. Bai. (2011). Effect of *Rhizobium* inoculation on the growth and yield of horsegram (*Dolichos biflorus* Linn). *Plant Archives*, 11(1): 97-99.

Khalimi, K.D. and N. Suprata. (2011). Induction of plant resistance against Soybean Stunt Virus using some formulations of *Pseudomonas aeruginosa*. *J ISSAAS*., 17: 98-105.

Martinez-Hidalgo, P. and A.M. Hirsch. (2017). The nodule microbiome: N_2 fixing rhizobia do not live alone. Review. *Phytobiomes*, 1: 70-82.

Masciarelli, O., A. Llanes and V. Luna. (2014). A new PGPR co-inoculated with *Bradyrhizobium japonicum* enhances soybean nodulation. *Microbiological Res*., 169: 609-615.

Mohamed, A.A. and M.A. Hassan. (2015). Evaluation of two chickpea (*Cicer arientinun* L.) cultivars in response to three *Rhizobium* strains at River Nile State, Sudan. *Merit Research Journal of Agricultural Science and Soil Science*, 3(5): 062-069.

Noreen, R., H.A. Shafique, S.A. Ali, Habiba, V. Sultana, J. Ara, S. Ehteshamul-Haque. (2016). Role of mungbean root nodule associated fluorescent *Pseudomonas* and rhizobia in suppressing the root rotting fungi and root knot nematode affecting chickpea (*Cicer arietinum* L.). *Pak. J. Bot*., 48: 2139-2145.

Noreen, R., S.A. Ali, K.A. Hasan, Habiba, F. Urooj, A. Tariq V. Sultana, J. Ara and S. Ehteshamul-Haque. (2019). Role of fluorescent *Pseudomonas* associated with root nodules of mungbean in the induction of nodulation by the rhizobia in mungbean. *Pak. J. Bot*., 51(3): (In press).

Noreen, R., S.A. Ali, K.A. Hasan, V. Sultana, J. Ara and S. Ehteshamul-Haque. (2015). Evaluation of biocontrol potential of fluorescent *Pseudomonas* associated with root nodules of mungbean. *Crop Protect*, 75:18-24.

Ntambo, M.S., I.S. Chilinda, A. Taruvinga, S. Hafeez, T. Anwar, R. Sharif, C. Chambi and L. Kies. (2017). The effect of rhizobium inoculation with nitrogen fertilizer on growth and yield of soybeans (*Glycine max* L.). *IJB* 10(3): p. 163-172.

Oswald, A., P.C. Velez, D.Z. D´avila and J.A. Pineda. (2010). Evaluating soil rhizobacteria for their ability to enhance plant growth and tuber yield in potato. *Ann. Appl. Biol*., 157: 259-271.

Park, M., C. Kim, J. Yang, H. Lee, W. Shin, S. Kim and T. Sa. (2005). Isolation and Characterization of diazotrophic growth promoting bacteria from rhizosphere of agricultural crops of Korea. *Microbiol. Res*. 160: 127-133.

Pepper, I.L. (2000). Beneficial and pathogenic microbes in agriculture. In*: Environmental Microbiology*. R. M. Maier *et al.* (Ed.). Academic Press. San Diego.

Rahman, A., V. Sultana, J. Ara and S. Ehteshamul-Haque. (2016). Induction of systemic resistance in cotton by the neem cake and *Pseudomonas aeruginosa* under salinity stress and *Macrophomina phaseolina* infection. *Pak. J. Bot*., 48: 1681-1689.

Saldana, G., V. Martinez-Alcantara, J.M. Vinardell, R. Bellogin, J.E. Ruiz-Sainz and P.A. Balatti. (2003). Genetic diversity of fast-growing rhizobia that nodulate soybean (*Glycine max* L. Merr). *Arch. Microbiol*., 180: 45-52.

Salih, S.H., S.A.M. Hamd and Y.M.I. Dagash. (2015). The effects of rhizobium, mycorrhizal inoculations and diammonium phosphate (DAP) on nodulation, growth and yield of soybean. *Universal Journal of Agricultural Research*, 3(1): P11-14.

Shafique, H.A., A. Rahman and F. Urooj. (2017). Role of *Trichoderma harzianum*, fluorescent *Pseudomonas* and *Rhizobia* in managing the root rot disease of tomato in soil amended with mustard cake. *Int. J. Biol. Res*., 5(1): 11-14.

Sharma, S.K., M.P. Sharma, A. Ramesh and O.P. Joshi. (2012). Characterization of zinc solubilizing *Bacillus* isolates and their potential to influence zinc assimilation in soybean seeds. *J. Microbiol. Biotechnol*., 22: 352-359.

Siddiqui, I.A. and S. Ehteshamul-Haque. (2001). Suppression of root rot-root knot disease complex by *Pseudomonas aeruginosa* in tomato: the influence of inoculums density, nematode population, moisture, and other plant associated bacteria. *Plant Soil*, 237: 81-89.

Susilowatl, A., A.T. Wahyudi, Y. Lestari, A. Suwanto and S. Wiyono. (2011). Potential *Pseudomonas* Isolated from Soybean Rhizosphere as Biocontrol against Soilborne Phytopathogenic Fungi. *HAYATI J. Biosci*., 18: 51-56.

Tran, V.T., T.T.P. Lien and C.H. Mau. (2015). Proteomic characterization of soybean leaf proteins from cultivar DT2000 with rust-resistant ability in Vietnam. *International Journal of Biosciences*, 6(2): 468-477.

Urooj, F., Farhat, H., Ali, S. A., Ahmed, M., Sultana, V., Shams, Z. I., ... & Ehteshamul-Haque, S. (2018). Role of endophytic penicillium species in suppressing the root rotting fungi of sunflower. *Pak. J. Bot*, *50*(4), 1621-1628.

Wilcox, J.R. and R.M. Shibles. (2001). Interrelationships among seed quality attributes in soybean. *Crop Science*, 41(1): 11-14.

Youseif, S.H., F.H. Abd El-Megeed, A. Ageez, Z.K. Mohamed, A. Shamseldin and S.A. Saleh. (2014). Phenotypic characteristics and genetic diversity of rhizobia nodulating soybean in Egyptian soils. *Eur. J. Soil Bio*., 60: 34-43.

Zhang, Y.M., X.H. Sui, Y.J. Li, Q.Q. Li and W.X. Chen. (2012). *Bradyrhizobium huanghuaihaiense* sp. nov., an effective symbiotic bacterium isolated from soybean (*Glycine max* L.) nodules. *I. J. Syst. Evo. Microbiol*., 62: 1951-1957.

Zuffo, A.M., P.M. Rezende, A.T. Bruzi, N.T. Oliveira, I.O. Soares, F.G.N. Geraldo, E.S.C. Bruno and L.O. Silva. (2015). Co-inoculation of *Bradyrhizobium japonicum* and. *Azospirillum brasilense* in the soybean crop. *Revista Brasileira de Ciências Agrárias*, 38(1): 87-93.

(Received February 2018; Accepted May 2018)