Int. J. Biol. Res., 5(2): 71-75, 2017.

RHIZOBIA SUPPRESS THE ROOT KNOT NEMATODE AND ROOT ROTTING FUNGI ON MUNGBEAN

Hafiza Farhat[1], Faizah Urooj[1], Hafiza Asma Shafique[1], Viqar Sultana[2] and Syed Ehteshamul-Haque[1]

[1]Agricultural Biotechnology & Phytopathology Laboratory, Department of Botany, University of Karachi, Karachi 75270, Pakistan
[2]Department of Biochemistry, University of Karachi, Karachi 75270, Pakistan

ABSTRACT

To check the rhizobial efficacy, fifteen isolates of *rhizobia* from mungbean (*Vigna radiata* L.) roots were studied for their activity against root knot nematode and root rotting fungi. *Rhizobial* cell free culture filtrates of this isolates inhibited egg hatching by showing significant nematicidal activity against *Meloidogyne incognita.* Antifungal activity of rhizobial isolates were checked against four common plant pathogens *viz., Macrophomina phaseolina, Rhizoctonia solani, Fusarium oxysporum* and *F. solani* caused growth inhibition of these fungi *in vitro.* In screen house experiments seven isolates of *rhizobia,* NFB-301, NFB-302, NFB-303, NFB-304, NFB-305, NFB-306 and NFB-307 were tested on mungbean plants. Most of the tested isolates significantly ($p<0.05$) increased plant growth parameters. Application of most isolates also suppressed infection of root rotting fungi.

KEYWORDS: *Rhizobia*, root rot fungi, root knot nematode, mungbean.

INTRODUCTION

Mungbean is an Asian crop and broadly grown in several countries such as Australia, continents of Africa and Asia (Yang *et al*., 2008). Inoculation of mungbean with *Rhizobium* increase growth, photosynthetic activity and production of dry matter (Thakur and Panwar, 1995*). Rhizobia* may be differentiated by their growth rate on many substrates in the form of fast and slow growth (Löhis and Hansen, 1921). The change made for the first time in the *rhizobial* nomenclature was the finding of *Bradyrhizobium* (Jordan, 1982). *Bradyrhizobium* strain, which make nodule in soybean plants, is recognized as *Bradyrhizobium japonicum*, that was the first identified group of *Bradyrhizobium* (Peter *et al.,* 1996).

Rhizobia may have certain mechanisms to control plant pathogens, these mechanisms are, Carrillo and Del Rosario (1992); Arora *et al*. (2001) struggle for iron to produce siderophores, Essalmani and Lahlou (2002) nutrient competition rate, Chakraborty and Purkayastha,(1984), production of antibiotics and nodule formation for promoting plant growth. Siddiqui and Mahmoud, (2001); Siddiqui *et al*. (2000a). *Rhizobia* have a capability to use in the form of biocontrol agent. Ehteshamul-Haque and Ghaffer, (1993).

Root rotting fungi mostly *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium* cause significant yield losses to several important food crops globally including mungbean (Bokhari *et al.,* 2014). Similarly, destructive nematodes known as root knot nematodes (*Meloidogyne* spp.) are widely distributed and attack on a several crops variety (Taylor and Sasser, 1978).The aim of the present study was to evaluate the rhizobial isolates, from the nodules of mungbean roots and their potential against fungi causing root rot and root knot nematode infecting mungbean.

MATERIALS AND METHODS

Rhizobial isolation from the nodules of mungbean root: For rhizobial isolation, roots samples containing nodules were collected from experimental field (Department of Botany) University of Karachi, Karachi. The samples were brought in laboratory and preserved at low temperature and isolates were obtained within 24 hours.

Roots were surface sterilized by placing them into 70% ethanol for round about 1 minute and after it in solution of 1% sodium hypochlorite for 3 min. Sterilized water was used to wash root nodules thoroughly and crushed in microfuge tube containing 100 µL of 15% glycerol. Suspension of ten µL was streaked on the surface of the medium of (YMA) yeast extract mannitol agar having congo red (Somasegaran and Hoben, 1994). White/cream colored gummy colonies grew after 5 days were purified. The isolates were initially identified based on cultural, morphological and biochemical tests using Bergey's manual of systemic bacteriology (Garrity *et al*., 2005).

***In vitro* activity of root rotting fungi:** To check antifungal activity in test bacterial strains, dual culture plate method was used (Noreen *et al.,* 2015). On one side of Czapek's Dox Agar (pH 7.2) test bacteria was streaked while on the other side of plates a disc of 5mm of fungus was placed. At 28^{o}C respective plates were incubated and after 3-7 days inhibition zone was measured.

Rhizobial cell free filtrate and juvenile mortality of nematode: Bacterial isolates were grown on YMA broth at 30^0C for round about 48 hours and at 300rpm centrifuged twice for round about 20 minutes. Cultural filtrates were collected in sterile beaker for further experiment. In a cavity blocks, suspension of 1 mL of newly hatched 2^{nd} stage Juvenile (10-15 juveniles) and bacterial cell free culture filtrate of 1mL was added and put at room temperature ± 5^oC. For each treatment 3 replicates were used and after 48hours juvenile mortality rate was counted.

Screen house experiments: All the treatments were conducted under greenhouse conditions in earthen pots in randomized completed block design. The soil used in this experiments were having a natural invasion of 3 to 6 per g sclerotia of *Macrophomina phaseolina* identified by wet sieving and serial dilution technique (Sheikh and Ghaffar1975). The seeds of sorghum were used in the form of baits for colonization of 5-10% *Rhizoctonia solani* (Wilhelm, 1955) and soil were also containing diverse population of 3000 cfu/g *Fusarium oxysporum* and *F. solani* (Nash and Snyder, 1962). In 1 kg pot soil, six seeds of mungbean were sown at the rate of 50mL/pot, rhizobial suspension (10^8 cfu/mL) was applied. Pots without treatments were served as control, while carbendazim used as positive control. Four seedlings per pot were left after thinning in every pot. *Meloidogyne incognita* suspension with 1000 eggs\J2 was added in each pot.

For determination of the efficiency of *rhizobia* against soil borne pathogens, plants removed from the pots after six weeks of inoculations of nematodes. They were washed thoroughly with water. Data were recorded in the form of growth parameters. For nematode infection, knots per root system were counted and incidence of root rot fungi recorded (Habiba *et al.,* 2016).

Analysis of data: The data were statistically analyzed using Analysis of Variance (ANOVA) and their means separated by using the least significant difference (LSD) following Gomez and Gomez (1984).

EXPERIMENTAL RESULTS

***In vitro* juvenile's mortality of root knot nematode:** Cultural filtrates of *rhizobia* caused differential mortality of nematodes within 48 hours. Culture filtrates of NFB-301, NFB-302, NFB-303, NFB-304, NFB-305, NFB-306, NFB-307, NFB-308, NFB-309, NFB-310, NFB-311, NFB-312, NFB-313, NFB-314 and NFB-315 caused 53.3, 51.6, 65, 58.3, 65, 70, 76.6, 70, 86.6, 68.3, 61.6, 63.3, 65, 73.3 and 78.4% juvenile mortality, respectively (Table 1).

Table1. *In vitro* growth inhibition of *Macrophomina phaseolina, Fusarium solani, Fusarium oxysporum, Rhizoctonia solani* and nematicidal activity of rhizobial isolates.

		Zone of inhibition for different fungi (mm)				Juvenile mortality after 48 h (%)
S. No.	Treatments	*F. oxysporum*	*F. solani*	*R. solani*	*M. phaseolina*	
	Control	_	_	_	_	30
A	NFB-301	13	7	10	16	53.3
B	NFB-302	43	12	16	25	51.6
C	NFB-303	26	20	36	19	65
D	NFB-304	35	21	32	29	58.3
E	NFB-305	37	12	18	31	65
F	NFB-306	41	0	33	0	70
G	NFB-307	24	13	18	31	76.6
H	NFB-308	19	9	29	10	70
I	NFB-309	17	18	16	13	86.6
J	NFB-310	0	13	35	18	68.3
K	NFB-311	15	9	0	12	61.6
L	NFB-312	17	18	21	19	63.3
M	NFB-313	14	11	32	20	65
N	NFB-314	22	13	17	0	73.3
O	NFB-315	20	14	19	0	78.4

Table 2. Effect of *rhizobial* isolates used as in soil drench on the infection of *Macrophomina phaseolina, Fusarium solani, Fusarium oxysporum* and *Rhizoctonia solani* on mungbean root.

Sr. No.	Infection (%)				
	Treatments	*F. solani*	*F. oxysporum*	*M. phaseolina*	*R. solania*
A	Control	56.2	62.5	*75*	*37.5*
B	Carbendazim	33.3	43.7	62.5	41.6
C	NFB-301	41.6	50	58.3	25
D	NFB-302	18.75	18.75	62.5	31.25
E	NFB-303	18.75	25	37.5	31.25
F	NFB-304	6.25	0	18.75	12.5
G	NFB-305	37.5	18.75	0	0
H	NFB-306	25	25	50	18.75
I	NFB-307	43.7	18.75	31.2	43.7

$LSD_{0.05}$ = Treatment = 15.1; Pathogen = 10.1

***In vitro* test of root rotting fungi:** Out of 15 isolates of *rhizobia* tested against root rotting fungi viz., *Macrophomina. phaseolina, Fusarium solani, F.oxysporum* and *Rhizoctonia solani*, maximum zone of inhibition against *Macrophomina phaseolina* was measured in NFB- 302, NFB-4 NFB-5 and NFB-7 which was more than 20mm. *S*imilarly, NFB-302, NFB-303, NFB-304, NFB-305, NFB-306, NFB-307 and NFB-314 produced zone of inhibition against *F. oysporum* more than 20mm,while NFB-303 and NFB-304 produced zone of inhibition more than 20mm against *F. solani.* Whereas, NFB-203, NFB-204, NFB-206, NFB-208, NFB-210, NFB-212 and NFB-213 produced zone of inhibition against *R. solani* more than 20mm (Table 1).

Screen house experiment: After six weeks of application of rhizobial isolates NFB-301, NFB-302, NFB-303, NFB-304, NFB-305, NFB-306, NFB-307, Carbendazim significantly ($p<0.05$) lowered the infection rate of *M. phaseolina*, *F. solani, F. oxysporum* and *R. solani* (Table 2). *M. phaseolina* and *R. solani* did not appear in NFB-305 treatment, while NFB-304 treated plants indicated no infection of *Fusarium oxysporum* (Table 2). Weight of root were significantly ($p<0.05$) increased by isolates NFB-302 and NFB-304 (Fig. 1). Height of mungbean plants were significantly ($p<0.05$) increased by isolated NFB-303, NFB-304 and NFB-305 (Fig. 2) while fresh weight of shoot were significantly ($p<0.05$) increased by NFB-301, NFB-303 and NFB-304 (Fig. 3). Root lengths were also significantly ($p<0.05$) increased by isolates NFB-302, NFB-303 and NFB-304 (Fig. 4). Maximum number of nodules were significantly ($p<0.05$) increased by isolates NFB-303 and NFB-6 (Fig. 5). Application of rhizobial isolates NFB-304 and NFB-305 caused reduction of nematode penetration in roots and gall formation on roots (Fig. 6).

DISCUSSION

Nitrogen fixing bacteria are associated with higher plants and provide them nitrogen. In this way suitable condition must provide them in such circumstances that plant gets full advantage (Stacy *et al.,* 1992, Walsh, 1995). The major group of mutual association with legumes is nitrogen-fixing bacteria (Sprent, 2001). In ecosystem, legumes play an important role (Van der Heijden *et al.,* 2006, Temperton *et al.,* 2007). *Rhizobia* are considered as important sources of nitrogen which enhance nitrogen source in plants. (Sprent and Sprent, 1990).

In this study fifteen isolates were obtained from mungbean root nodule which showed significant antifungal activity against common pathogenic fungi. The ability of *rhizobia* to suppress many soilborne plant pathogens (Ehteshamul-Haque and Ghaffar, 1992) enhances the value of *rhizobia* other than their use for nitrogen fixation. *Rhizobia* had showed significant results to control the root rotting fungi on both non leguminous and leguminous plants under field condition (Noreen *et al.,* 2015). Dutta *et al.* (2008) recognized *rhizobia* as plants growth-promoter and they are known for the production of enzyme involved in defense system in pigeon pea Johnson and Bentley (1991) find out the increase production rate of alkaloid in lupines leaves due to symbiotic nitrogen fixation. Photosynthetic reduction due to herbivory may alter the process of nitrogen fixation and *rhizobia* may produce a benificial effects on the growth of plants (Johnson and Bentley, 1991). Parasitic nematodes, termed as (hidden enemies) are the most destructive pathogens that cause major losses of crop plants. In this study rhizobial isolates used, as a soil drench, significantly prevented the infection of root knot nematode. It is known that *rhizobia*, besides nitrogen fixation can also suppress nematode attack (Ehteshamul-Haque *et al.*, 2007). Application of *rhizobia* as biocontrol agent, to control soil-borne pathogens, seems to be potentially useful besides their use in biological nitrogen fixation.

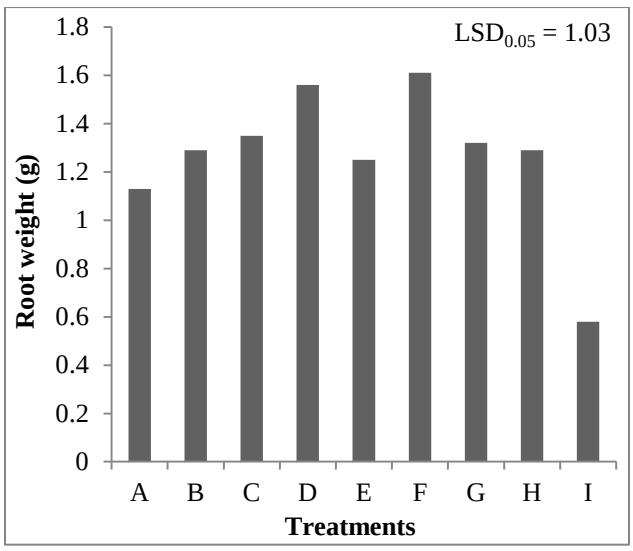

Fig. 1. Effect of rhizobial isolates on root weight of mungbean.

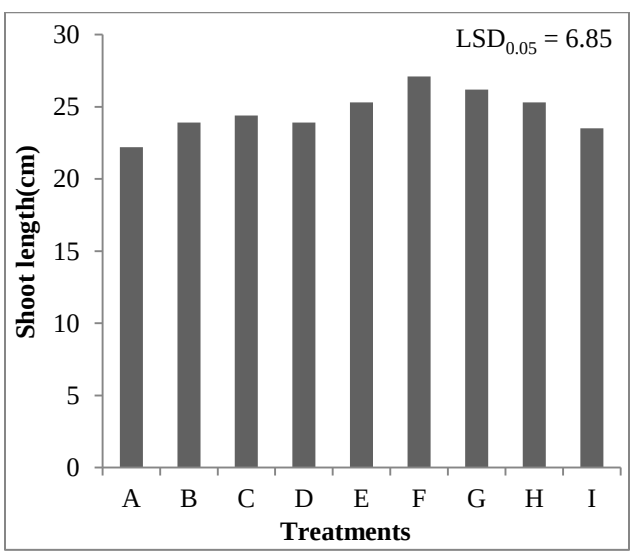

Fig. 2. Effect of rhizobial isolates on shoot length of mungbean.

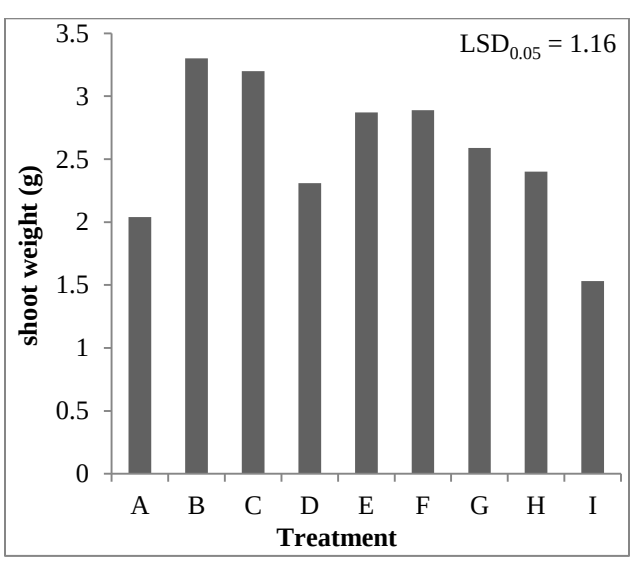

Fig. 3. Effect of rhizobial isolates on shoot weight of mungbean.

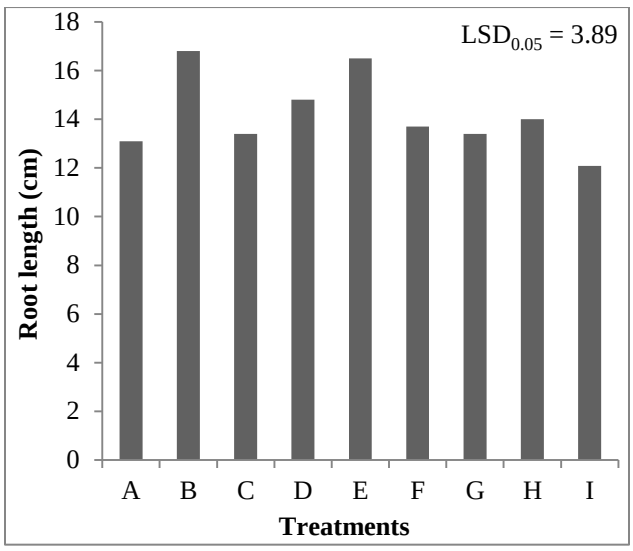

Fig. 4. Effect of rhizobial isolates on root length of mungbean.

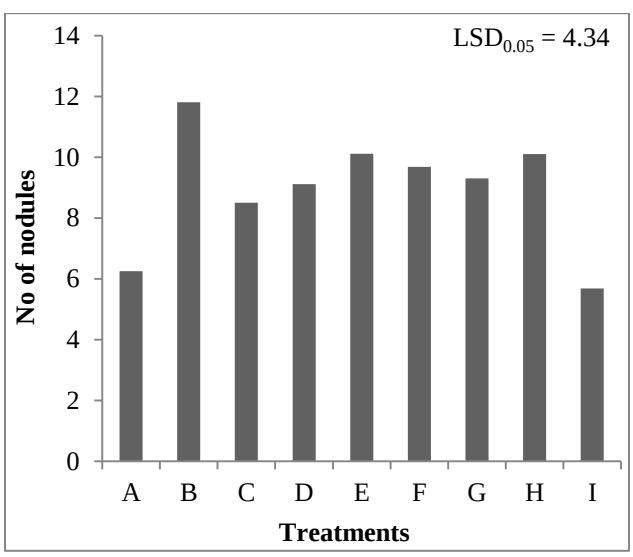

Fig. 5. Effect of rhizobial isolates on nodule of mungbean.

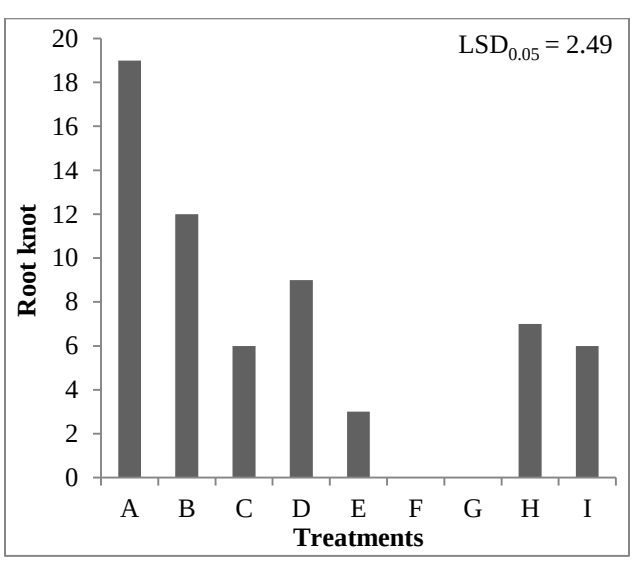

Fig. 6. Effect of different isolates of rhizobia on nematode infection (number of knots per root system).

REFERENCES

Arora N.K., S.C. Kang and D.K. Maheshwari. (2001). Isolation of siderophores-producing strains of *Rhizobium meliloti* and their biocontrol potential against *Macrophomina phaseolina* that causes charcoal rot of groundnut. *Current Science*, 81: 673-677.

Bokhari, A., A. Alkan, R. Dogan, M. Diaz-Aguiló, F. De Leon, D. Czarkowski and R.E. Uosef. (2014). Experimental determination of the ZIP coefficients for modern residential, commercial, and industrial loads. *IEEE Transactions on Power Delivery*, 29(3): 1372-1381.

Carrillo G.C. and V.M. Del Rosario. (1992). Comparative study of siderophore like activity of *Rhizobium phaseoli* and *Pseudomonas fluorescens. Journal of Plant Nutrition,* 15: 579-590.

Chakraborty, U. and R.P. Purkayastha. (1984). Role of rhizobitoxine in protecting soybean roots from *Macrophomina phaseolina* infection. *Canadian Journal of Microbiology*, 30(3): 285-289.

Dutta, S., A. K. Mishra, and B.D. Kumar. (2008). Induction of systemic resistance against Fusarial wilt in pigeon pea through interaction of plant growth promoting *rhizobacteria* and *rhizobia*. *Soil Biology and Biochemistry*, 40(2): 452-461.

Ehteshamul-Haque, S. and A. Ghaffar. (1993). Use of *rhizobia* in the control of root rot diseases of sunflower, okra, soybean and mungbean. *Journal of Phytopathology*, 138(2): 157-163.

Ehteshamul-Haque, S. V. Sultana, J. Ara and M. Athar. (2007). Cultivar response against root-infecting fungi and efficacy of *Pseudomonas aeruginosa* in controlling soybean root rot. *Plant Biosystems*, 141(1): 51-55.

Ehteshamul-Haque, S.R.Y. Hashmi and A. Ghaffar. (1992). Biological control of root rot disease of lentil. *Lens*, 19(2):43-45.

Essalmani, H. and H. Lahlou. (2002). *In vitro* antagonistic activity of some microorganisms towards *Fusarium oxysporum* f. sp. lentis (French). *Cryptogamie-Mycologie*, 23(3): 221-234.

Garrity, A. (2005). Validation of publication of new names and new combinations previously effectively published outside the IJSEM. *Int J Syst Evol Microbiol.*, 55: 2235-2238.

Gomez, K.A. and A.A. Gomez. (1984). *Statistical Procedures for Agricultural research*. John Wiley & Sons.

Habiba, R. Noreen, S.A. Ali, V. Sultana, J. Ara and S. Ehteshamul-Haque. (2016). Evaluation of biocontrol potential of epiphytic fluorescent *Pseudomonas* associated with healthy fruits and vegetables against root rot and root knot pathogens of mungbean. *Pak. J. Bot.,* 48: 1299-1303.

Johnson, N. D. and B.L. Bentley. (1991). Symbiotic N2-fixation and the elements of plant resistance to herbivores: lupine alkaloids and tolerance to defoliation. Wiley, pp. 45-63 187.95.

Jordan, D.C. (1982). Transfer of *Rhizobium japonicum* Buchanan 1980 to *Bradyrhizobium* gen. nov., a genus of slow-growing, root nodule bacteria from leguminous plants. *International Journal of Systematic and Evolutionary Microbiology*, 32(1): 136-139.

Löhis, F. and R. Hansen. (1921). Nodulating bacteria of leguminous plant. *J. Agric. Res*., 20: 543-556.

Nash, S.M. and W.C. Snyder. (1962). Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soils. *Phytopathology*, 52(6): 101-116.

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 Protection*, 75: 18-24.

Peter, J., W. Young and K.E. Haukka. (1996). Diversity and phylogeny of *rhizobi*a. *New Phytologist*, 133(1): 87-94.

Sheikh, A.H. and A. Ghaffar. (1975). Population study of the sclerotia of *Macrophomina phaseolina i*n cotton fields. *Pak. J. Bot*., 23(5): 123-135.

Siddiqui, I.A., F. Aleem, M.J. Zaki and S.S. Shaukat. (2000). Control of *Meloidogyne javanica* by the nematophagus fungi. *International Journal of Nematology*, 10(2): 219-222.

Siddiqui, I.A., S. Ehteshamul-Haque, M.J. Zaki and A. Ghaffar. (2000a). Greenhouse evaluation of *rhizobia* as biocontrol agent of root infecting fungi in okra. *Acta Botanica*, 53: 13-22.

Siddiqui, Z.A. and I. Mahmoud. (2001). Effects of *rhizobacteri*a and root symbionts on the reproduction of *Meloidogyne javanica* and growth of chickpea. *Bioresource Technology*, 79: 41-45.

Somasegaran, P. and H.J. Hoben. (1994). *Quantifying the growth of rhizobia.* In *Handbook for Rhizobia* Springer, New York, NY, pp. 47-57.

Sprent, J.I. (2001). Nodulation in legumes. Royal Botanic Sprent, J. I., J. M. Sutherland, and S. M. de Faria. 1987. Some aspects of the biology of nitrogen-fixing organisms. Philosophical Transactions of the Royal Society of London B, *Biological Sciences* 317: 111-129.

Sprent, J.I. and P. Sprent. (1990) *Nitrogen fixing organisms: Pure and Applied Aspects*, Chapman and Hall, London, pp. 256.

Stacy, A.W., M.D. Newcomb and P.M. Bentler. (1992). Interactive and higher-order effects of social influences on drug use. *Journal of Health and Social Behavior*, 53(4): 226-241.

Taylor, A.L. and J.N. Sasser. (1978). *Biology, identification and control of root-knot nematodes. North Carolina State University Graphics*, 23(6): 111-121.

Temperton, V.M., P.N. Mwangi, S.M. cherer-Lorenzen, B. Schmid and N. Buchmann. (2007). Positive interactions between nitrogen-fixing legumes and four different neighbouring species in a biodiversity experiment. *Oecologia*, 151(2): 190-205.

Thakur, A.K. and J.D.S. Panwar. (1995). Effect of *Rhizobium*-VAM interactions on growth and yield in mungbean (*Vigna radiata* (L.) Wilczek) under field conditions. *Indian Journal of Plant Physiology*, 65(7): 204-218.

Van Der Heijden, M., G.R. Bakker, J. Verwaal, T.R. Scheublin, M. Rutten, R. Logtestijn and C. Staehelin. (2006). Symbiotic bacteria as a determinant of plant community structure and plant productivity in dune grassland. *FEMS microbiology ecology*, 56(2): 178-187.

Walsh, K.B. (1995) Physiology of the legume nodule and its response to stress", *Soil Biol. Biochem.*, 27: 637-655.

Wilhelm, S. (1955). Longevity of the *Verticillium* wilt fungus in the laboratory and field. *Phytopath*., 45: 180-181.

Yang, J.K., T.Y. Yuan, W.T. Zhang, J.C. Zhou and YG. Li. (2008). Polyphasic characterization of mungbean (*Vigna radiata* L.) *rhizobia* from different geographical regions of China. *Soil Biol. Biochem.*, 40(7): 1681-1688.

(Received 25 July 2017; Accepted 4 September 2017)