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REGULAR ARTICLE

CO-INOCULATION STUDY OF *BRADYRHIZOBIUM JAPONICUM* AND *ASPERGILLUS NIGER* IN SOYBEAN FOR NITROGEN FIXATION

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ABSTRACT

Present study was aimed to evaluate the liquid inoculation of ten *Bradyrhizobium japonicum* with fungi *Aspergillus niger* (plant growth promoting) in pot experiments for determination of nitrogen fixation potential. Commonly grown JS-335 cultivar of Soybean around Ujjain region was used as host plant. Nodule number, shoot length, root length and their dry weight were taken as criteria for nitrogen fixation. Inoculation in pot along with fertilizer significantly increase the nodulation, shoot and root length at 5% of probability over inoculation of only *B. japonicum* and uninoculated. Total 12 treatments (10 tests + 2 controls) with three replicates were set and total 36 pots were arranged. Results revealed that *B. japonicum* increases the nitrogen fixation in combination with *A. niger* and induces the plant growth directly like phosphate solubilization, through IAA production, ammonia production and indirectly by inhibiting the pathogenic fungi. Co-inoculation was found suitable and effective over single inoculums. The present study supports that liquid inoculum of more than one organism results in increase in nodulation, and can effectively increase the crop productivity.

Keywords: Yeast Extract Mannitol Broth, Potato Dextrose Broth, Nodule Occupancy Per Plant

INTRODCUTION

In present scenario where use of chemical fertilizers is excessive, the need of eco-friendly and cost-effective agricultural practices are the prime attention (**Dube and Maheshwari, 2006**). The economic and environmental costs of the heavy use of chemical fertilizers in agriculture are of a global concern and there is an urgent need to seek some alternatives to fertilizers. The ultimate option is to go for sustainable agriculture practices. Soybean has played a significant contribution to yellow revolution in India (**Chauhan and Joshi, 2005**), and as a food plant it forms a part of routine diet of the people (**Tiwari, 1999**). Symbiotic bacteria such as *Rhizobium*, *Bradyrhizobium*, and *Sinorhizobium* belonging to the group rhizobia (from the Latin 'root living') play an important role in the nutrition of leguminous plants by fixing atmospheric nitrogen in root nodules (**Howieson *et al*., 2000a; Zakhia *et al*., 2004**). Most of the strains of *Bradyrhizobium* are associated with soybean species (**Sameshima *et al*., 2003; Giongo *et al*., 2008**). For economically viable and environmentally prudent farming practices, successful management of nitrogen inputs through Biological Nitrogen Fixation (BNF) with the help of legumes is essential (**Bohlool** and **Schmidt, 1968; Vance** and **Graham, 1995**). In context of increasing international concern for food and environmental quality, the use of PGP (Plant Growth Promoting) microbes for reducing chemical inputs in agriculture is a potentially important issue. PGP microbes have been used in various crops for seed emergence, enhanced growth, crop yield and biological control (**Day *et al*., 2004; Thakuria *et al*., 2004; Herman *et al*., 2008**). Co-inoculation studies with plant growth promoting rhizobacteria (PGPR) and *Bradyrhizobium* have shown increased plant nodulation and nitrogen fixation under normal growth conditions (**Verma *et al*., 1986; Li** and **Alexander, 1988**).

The objective of our study was to evaluate the possible role of indigenous PGP fungal and *B.japonicum* isolates on plant growth in terms of nitrogen fixation.

MATERIAL AND METHODS

Isolation and identification of Bradyrhizobia

B. japonicum was isolated from the freshly nodules collected from the field and identified by different morphological and biochemical identification following **Buchanan** and **Gibbons (1984).** For nodule collection, Soybean plants were excavated carefully avoiding any injury and were brought in the laboratory. Root nodules were washed under a gentle stream of water. Young, healthy and pinkish root nodules were selected for the bacterial isolation. Isolation of the bacteria was performed following **Subba Rao (1984)**.

Seed germination

Seeds of JS-335 Soybean cultivar procured from the JawaharLal Nehru Agriculture Centre, Ujjain and were surface-sterilized with 0.1% $HgCl_2$. After rinsing with water sufficiently, seeds were transferred in Petri dishes (10 cm) lined with two circles of cellulose filter papers, moistened with about 4 ml distilled water. Twenty surface sterilized seeds were placed in the sterilized Petri plates and incubated at 30°C. Apparently healthy germinated seeds were selected and transferred in the previously prepared pots. In each pot, 15 seeds were placed about 2-3 cm deep in soil.

Pot arrangement

Soil for the pots was colleted from the fields in polythene bags and sterilized in autoclave at 15Lb/inch^2. Six kilograms of soil was filled in the earthen-pots already treated with 5% $CuSO_4$ solution. Various isolated and identified strains of *B. japonicum* that were isolated from root nodules of local soybean cultivar JS-335 were grown in the YEM (yeast extract mannitol) broth (HiMedia Lab., Mumbai) and *A. niger Aspn1* strain, isolated from rhizospheric soil of same soybean cultivar (JS-335) following **Johnson *et al.* (1959)** and identified using A Manual of Soil Fungi by **Gillman (1962)**, was grown in potato dextrose broth. After proper growth of both the microbes (1×10^8cells ml^{-1} for bacteria and 1×10^4cells ml^{-1} fungi, measured using Haemocytometer), a liquid inoculum (culture) was prepared and mixed with soil in the ratio of 1: 60. Sufficient amount of water and fertilizer were added. Seedlings were observed appropriately for development and watered whenever needed. After 32 days of normal growth (period for onset of flowering in JS-335 cultivar) ten plants from each pot were used for the estimation of shoot length, shoot dry weight, root length, root dry weight and numbers of root nodules per plant. For dry weight estimation, shoot and root samples were dried overnight at 72°C.

Statistical analyses

The statistical analyses were done using **Gomez** and **Gomez (1968)**, **Zar (2004),** and SPSS (Statistical Package, Version 10.0). The variables were subjected to ANOVA and were tested for significance at $P<0.05$ and $P<0.01$ level.

RESULTS AND DISCUSSION

Nitrogen fixation is one of most important biological processes on earth (**Graham, 2008**) and *Rhizobium* inoculation of legumes is one of the success stories of world agriculture (**Herridge, 2008**). The response of inoculum with respect to nodule formation (NOPP)/ root and shoot length and their dry weight was reflected in dry matter production as well. In the present study, Table-1 and Table-2 reveal the status and effect of treatment of various indigenous bacterial (*B. japonicum*) co-inoculated with *A. niger Aspn1* on number of root nodules, shoot and root length, and their dry weight respectively. After 32 days of normal growth with different treatments of cultivar JS-335, plantlets were removed at the stage of flowering initiation. The shoot dry weight of plant harvested in floral initiation is the generally accepted criteria for nitrogen fixing effectiveness, but nodule number and dry weight may also be employed for more refined evaluation (**Somasegaran** and **Hoben, 1985**).

Table 1 Effect of co-inoculation of *B. japonicum* and *A. niger Aspn1* on number of root nodules, length of shoot and root in JS-335 soybean cultivar after 32 days of sowing in Black Cotton soil.

***B. japonicum* Isolates + *A. niger* Aspn1**	**No. of root nodules**	**Shoot length (cm)**	**Root length (cm)**
B1	23.50±2.3	14.32±2.5	11.32±1.60
B2	24.60±1.6	14.25±1.50	11.56±2.30
B3	26.50±3.3	14.6±2.30	11.23±2.10
B4	26.15±2.4	13.95±2.8	10.2±1.30
B5	25.00±2.8	13.24±1.8	10.2±0.9
B6	22.00±1.5	14.02±2.0	11.04±2.20
B7	24.50±3.6	13.56±1.8	10.28±1.6
B8	26.14±2.9	13.25±3.2	10.36±1.0
B9	22.50±2.7	13.45±2.7	11.02±2.80
B10	25.25±3.2	13.64±4.1	11.3±2.60
Control 1 (Uninoculated)	06.75±2.3	4.52±1.5	3.24±0.80
Control 2 Inoculated with *B. japonicum* only	15.60±3.4	10.31±3.2	8.86±3.0

B1, B2, B3…..B10, different isolates of *B. japonicum* co-inoculated with *A. niger Aspn1*

Control-1 (Seedlings with no bacterial and fungal inoculum, grown only with distilled water)

Control-2 (Seedlings inoculated with only *B. japonicum* inoculum) ± Standard Deviation.

Table 2 Effect of co-inoculation of *B. japonicum* and *A. niger Aspn1* on root nodules, dry weight of shoot and root in JS-335 soybean cultivar after 32 days of sowing in Black Cotton soil

***B. japonicum* Isolates + *A. niger* Aspn1**	**Root nodules dry weight (g)**	**Shoot dry weight (g)**	**Root dry weight (g)**
B1	1.38±0.25	0.92±0.18	0.72±0.12
B2	1.41±0.21	0.98±0.16	0.75±0.14
B3	1.63±0.14	0.99±0.09	0.72±0.16
B4	1.46±0.23	0.89±0.10	0.69±0.12
B5	1.4±0.11	0.87±0.23	0.68±0.18
B6	1.29±0.09	0.91±0.17	0.71±0.06
B7	1.41±0.19	0.89±0.13	0.68±0.10
B8	1.48±0.15	0.87±0.05	0.69±0.08
B9	1.30±0.10	0.88±0.08	0.69±0.06
B10	1.52±0.26	0.88±0.2	0.69±0.10
Control 1 (Uninoculated)	0.54±0.08	0.05±0.02	0.02±0.009
Control 2 Inoculated with *B. japonicum* only	0.86±0.15	0.71±0.073	0.57±0.08

B1, B2, B3…..B10, different isolates of *B. japonicum* co-inoculated with *A. niger* Aspn1

Control-1 (Seedlings with no bacterial and fungal inoculum, grown only with distilled water)

Control-2 (Seedlings inoculated with only *B. japonicum* inoculum) ± Standard Deviation.

Root nodules and dry weight

As evident from the Table-1, combination of *A. niger Aspn1* and the bacterial isolates increased number of root nodules when compared with control treatments. There is a strong positive relationship between number and dry weight of nodules and yield and per plant in soybean (**Roy** and **Mishra, 1975; Tiwari** and **Agrawal, 1977**). **Tarafdar** and **Rao** (**2001**), also reported positive interaction between *Rhizobium* and fungi inoculation on nodulation. In our study, there was a significant difference among isolates over controls was observed. However, the response of co-inoculation treatment varied with different bacterial strains. Based on the nodulation, we have grouped the isolates as excellent for more than 25 nodules per plant, very good for 24-25 nodules per plant while less than 24 nodules grouped as good. Therefore, isolate B3, B4 and B8 shows excellent nodulation, isolate B2, B5, B7 and B10 come under very good nodulation category while other isolates viz., B1, B6 and B9 were showing good nodulation (Table-1). Further, bacterial isolates with *A. niger* significantly increase the dry weight of root nodules over both the control treatments (Table 2). **Abril *et al.* (1997)** and **Nandasena *et al.* (2001)** also demonstrated that inoculation of soybean with native and well-adapted rhizobia resulted in a better response than inoculation with exotic, non-adapted strains. **Rastogi *et al.* (1981)** concluded that there was a significant increase in number of root nodule and dry matter of plants when *Rhizobium* culture was applied to the seedlings. Similar results were obtained by **Abaidoo *et al.* (2000)** with native *Bradyrhizobium* strains where increase in nodulation was observed in host plants. Several workers have demonstrated that inoculation with indigenous *Bradyrhizobium* strains favours the formation of nodules on primary root (**Milic *et al.*, 1992**; **Soliman *et al.*, 1996**). **Bhat *et al.* (2011**) also reproduce the data on nodulation and revealed that the inoculation with *Rhizobium* along with fungi significantly increased the nodule dry weight per plant. There was a strong positive correlation between number of root nodules and crop yield which suggest that optimization of root nodulation by inoculating compatible and effective *B. japonicum* strains significantly increase the soybean crop yield.

Shoot length and shoot dry weight

Effect of the bacterial and fungal combination was also studied on the shoot length and shoot dry weight (Table-1 and 2). Increase in shoot length was significantly higher in all the bacterial isolates over controls in combination with *A. niger*. Similar results were obtained in the case of shoot dry weight. The reason behind such type of results might be the production of some kind of plant growth promoting phytohormones by the fungal isolate and synergistic effect with *B. japonicum* (**Sevilla *et al.*, 2001; Kumutha *et al.*, 2004; Dudde** and **Raut, 2005)**. Isolates producing IAA have stimulatory effect on the plant growth. When the crop is inoculated with the isolates capable of IAA production, significant increase in uptake of N, P, K, Ca and Mg was reported by **Farzana** and **Radizah (2005)**. According to **Fuhrmann** and **Wollum (1989)**, some plant growth promoting microorganisms may promote plant growth indirectly by affecting symbiotic N_2 fixation, nodulation and shoot growth. Many researches confirmed that inoculation of effective rhizobial strains increase plant height (shoot) and dry matter production as well as seed yield. While in few other cases where certain rhizobial cultures have been reported, to exhibit better nodulation and to produce considerable increase in shoot and root length as well as shoot and root weight of seedlings in comparison to control (**Brar** and **Lal, 1991; Thakur** and **Panwar, 1995; Provorov *et al*., 1998; Sharma, 2001; Anjum *et al*., 2006; Mansoor, 2007**). **Chanway *et al*. (1989**) and **Yanni (1992)** worked with native rhizobial strains and obtained similar kind of results where increase in nodulation and shoot length was observed in field trial as well as in Leonard Jar assembly. Results of present study come in agreement with the observations of **Harper *et al*. (1997)** and **Ito *et al*. (2008)** that expression of nodulation and increase in shoot is directly related to the *Rhizobium* inoculum used. In present study, all ten isolates showed increase in shoot length and shoot dry weight over controls. Although, there was no significant difference observed in shoot length between the ten bacterial isolate treatments but a significant difference was observed when compared to respective controls (Table-1). Similar results have been reported by **Provorov *et al*. (1998)**, **Hayat *et al*. (2008)**, and **Adeghipour *et al*. (2010)**.

Root length and root dry weight

A. niger showed significant increase in root length and dry weight when co-inoculated with *B. japonicum* isolates (Table-1 and 2). **Dhami** and **Prasad (2009)** concluded that inoculation of *Rhizobium* increase the accumulation of N in the root and results in increase in root length and also reported that nitrogen content in soil of inoculated experimental pots increased up to 13-33%. It is known that indigenous rhizobial populations fix N_2 with different efficiency depending of their density and activity and affect the root part and nodulation (**Mpepereki *et al*., 2000; Musiyiwa *et al*., 2005**). Same type of results were obtained when a field experiment was conducted with soybean to find out the effect of inoculation and scheduling of irrigation on nodulation, accumulation and redistribution of nitrogen in plant parts specially root part (**Konde *et al*., 1998; Pahalwan** and **Tripathi 1999; Hazarika *et al*., 2000; Kumutha *et al*., 2004).** Therefore, higher availability of nitrogen, significantly increased uptake, dry matter accumulation, translocation of nutrients during reproductive stage which in turn improved yield attributes that affected the grain and straw yield at the end (**Patel *et al*., 1988; Kumar *et al*., 2002; Sharma *et al*., 2003**). Though nitrogen status and its partitioning in crop plants was not studied in present work but possibly it was at par with the reported earlier findings that is reflected in over all growth of soybean crop.

CONCLUSION

From all six parameters tested in the present study, it is clear that the combination of *B. japonicum and A. niger* is showing an increased nitrogen fixation. It is clearly revealed that seedling growth in soybean can be enhanced by co-inoculating the seeds with the effective rhizobial strains along with plant growth promoting fungi. The microbial strains should preferably be indigenous ones.

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REFERENCES

ADEGHIPOUR, O. – MONEM, R. – TAJALI, A.A. 2010. Production of Mungbean (*Vigna radiata* L.) as affected by nitrogen and phosphorus fertilizer application. In *J. Appl. Sci.* Vol. 10, 2010, p. 843-847.

ALI, A. – MALIK, M.A. – CHAUDHRY, M.A. – SIDDIQUE, M.A. – RAFIQUE, M. 1999. In growth and yield response of mungbean (*Vigna radiata* L.) to different seed rates and levels of phosphorous. *Pak. J. Biol. Sci.,* Vol. 2, 1999, p. 879-880.

ANJUM, M.S. – AHMED, Z.I. – RAUF, C.A. 2006. Effect of *Rhizobium* inoculation and nitrogen fertilizer on yield and yield components of mungbean. In *Int. J. Agric. Biol*. Vol. 2, 2006, p. 238-240.

BHALLA, G.S. - SINGH, G. 1997. Recent developments in Indian agriculture, a state level analysis. In *Econom. & Political Weekly.* Vol. 29, 1997, p. A 2-18.

BHAT, M.I. - BANGROO, S.A. - TAHIR, A.- YADAV, S.R.S. – AZIZ, M.A. 2011. In Combined Effects of *Rhizobium* and *Vesicular Arbuscular* Fungi on Green Gram (*Vigna radiata* L. Wilczek) under Temperate Conditions. *Research Journal of Agricultural Sciences*,. Vol. 2, 2011, 1, p. 17-20.

BOHLOOL, B.B. - SCHMIDT E.L. 1968. Nonspecific staining: its control in immune-fluorescence examination of soil. In *Science.* Vol. 162**,** 1968, p. 1012-1014.

BRAR, J.S. – LAL, P.B. 1991. Effect of *Rhizobium* inoculation, phosphorus and molybdenum on yield and its components in mungbean. In *Indian Agriculturist* . Vol.35, 1991, p. 67–69.

CHANWAY, C.P. – HYNES, R.K. – NELSON, L.M. 1989. Plant growth promoting rhizobacteria: effects on growth and nitrogen fixation of lentil and pea. In *Soil Biol. Biochem.* Vol. 21, 1989, p. 511-517.

DUBE, R.C. – MAHESHWARI, D.K. 2006. In A text book of Microbiology (Ed II) 2006, 506-512, p.

DUDDE, K.B. – RAUT, R.S. 2005. Combined effects of *Rhizobium* and VA-mycorrhiza inoculation on groundnut. In *Journal of Soils and Crops* Vol. 15, 2005, 2, p. 315-318.

FARZANA, Y. – RADIZAH, O. 2005. Influence of rhizobacterial inoculation on growth of the sweet potato cultivar. In *On Line Journal of Biological Science.* Vol. 1 2005, no. 3, p. 176-179.

FUHRMANN, J.J. - WOLLUM, A.G. 1989. Nodulation competition among *Bradyrhizobium japonicum* strains as influenced by rhizosphere bacteria and iron availability. In *Biology and Fertility of Soils.* Vol.7, 1989, no. 2, p. 108–112.

GILMAN, J.C. 1956. In A manual of Soil fungi (II eds). Oxford and IBH publishing Co. 1956, p. 220-230.

GOMEZ, K.A. – GOMEZ, A.A. 1984. In Statistical procedure for agricultural research. John Wiley and Sons, 1984, NY 178-182, 301-309, p. 368-372.

GRAHAM, P.H. 2008. Ecological of the root-nodule bacteria of legumes. In Dilworth, M. J, James, E. K, Sprent, J. I and Newton, W.E. (eds.) Nitrogen-fixing Leguminous Symbioses. Nitrogen Fixation: Orgins, Application. Volume 7. Published by Sprinker, 2008, p. 23-43.

HARPER, J.E. – CORRIGAN, K.A. – BARBERA, A.C. - ABD-ALLA, M.H. 1997. Hyper-nodulation of soybean, mung bean, and hyacinth bean is controlled by a common shoot signal. In *Crop Science.* Vol. 37, 1997, p. 1242–1246.

HAYAT, R. - ALI, S. - IJAZ, S.S. – CHATHA, T.H. – SIDDIQUE, M.T. 2008. Estimation of N_2-fixation of mung bean and mash bean through xylem ureide technique under rainfed conditions. In *Pak. J. Bot.* Vol. 40, 2008, p. 723-734.

HERRIDGE, D.F. 2008. Inoculation technology for legumes. In: Dilworth MJ, James EK, Sprent JI, Newton WE (Eds.), Leguminous Nitrogen-fixing Symbioses. Kluwer, Netherlands, 2008, p. 77–81.

ITO, S. – KATO, T. – OHTAKE, N. – SUEYOSHI, K. - OHYAMA, T. 2008. The autoregulation of nodulation mechanism is related to leaf development. In *Plant Cell Physiology.* Vol. 49, 2008, p.121–125.

KUMAR, R. – GUPTA, P.P. – JALALI, B.L. 2001. Impact of VAM, *Azotobacter* and *Rhizobium* on growth and nutrition of cowpea. In *Journal of Mycology and Plant Pathology.* Vol. 31, 2001, no. 3, p. 38-41.

KUMUTHA, K. – SEMPAVALAN, J. – SANTHONAKRISHNAN, P. 2004. Effect of insoluble phosphate and dual inoculation on soybean. In Biofertilizer technology Eds. S. Kannaiyan, K. Kumar and K. Govindaraj and scientific Publishers (India) Jodhpur, 2004 p. 354-358.

LI, D.M. – ALEXANDER, A. 1988. Co-inoculation with antibiotic-producing bacteria to increase colonization and nodulation by rhizobia. In *Plant and Soil.* Vol. 108, 1988, p. 211-219.

MILLIC, V. – SARIC, Z. – MRKOVACKI, N. - VOROSBARANYI L. 1992. *Braydrhizobium japonicum* capacity to synthesize growth regulators affecting nodulation and nitrogen uptake by soybean. In *Mikrobiologija Beograd*. Vol. 28, 1992, no. 2, p. 145-152.

MPEPEREKI, S. - JAVAHERI, F. – DAVIS, P. – GILLER, K.E. 2000. Soybeans and sustainable agriculture: promiscuous soybeans in Southern Africa. In *Field Crops Res*. Vol. 65, 2000, p.137-149.

MUSIYIWA, K. – MPEPEREKI, S. – GILLER, K.E. 2005. Symbiotic effectiveness and host ranges of indigenous rhizobia nodulating promiscuous soybean varieties in Zimbabwean soils. In *Soil Biol. Biochem.* Vol. 37, 2005, p. 1169-1176.

NADEEM, M.A. – AHMAD, R. – AHMAD, M.S. 2004. Effect of seed inoculation and different fertilizer levels on the on the growth and yield of mungbean (*Vigna radiata* L.). In *J. Agron*. Vol. 3, 2004, p. 40-42.

PATEL, R.R. – PATEL, J.R. – SONANI, V.V. – SASANI, G.V. 1988. Effect of inoculation, N and P fertilization on yield and yield attributes of green gram var. Gujarat-1 (*Vigna radiate* L. Wilczek). In *Gujarat Agricultural University Research Journal. Vol.* 14, 1988, 1p. 17-22.

PROVOROV, N.A. – SAIMNAZAROV, U.B. – BAHROMOV, I.U. – PULATOVA, D.Z. – KOZHEMYAKOV, A.P.- KURBANOV, G.A. 1998. Effect of rhizobia inoculation on the seed (herbage) production of mungbean (*Phaseolus aureus* Roxb.) grown at Uzbekistan. In *J. Arid Environ*. Vol. 39, 1998, p. 569-575.

RAHMAN, M.A. – ISLAM, N. – ISLAM, A. – HASSAN, M.K. – TALUKDER, M.M.R. 2002. Yield performance of Mungbean (*Vigna radiata* L. Wilczck) cv. Barimung-4 as Influenced by *Rhizobium* inoculation and NPK Fertilizer. In *Pak. J. Biol. Sci*. Vol. 5, 2002, p. 146-148.

SHARMA, C.K. – SARMA, H.K. 1999. Effect of different production factors on growth, yield and economics of mungbean (*Vigna radiata* L.). In *Indian J. Hill Farming.* Vol. 12, 1999, p. 29-31.

SEVILLA, M. – BURRIS, R.H. – GUNAPLA, N. – KENNEDY, C. 2001. Comparison of benefit to sugarcane plant growth and 15 N_2 incorporation following inoculation of sterile plants with *Acetobacter diazotrophicus* wildtype and Nif-mutant strains. In *Mol. Plant-Micr. Inter*. Vol. 14, 2001, p. 358-366.

SHARMA, S. - UPADHYAYA, R.G. - SHARMA, C. – RAMESHWAR, R. 2003. Response on growth, physiological parameters and yield of *Vigna radiate* L. Wilczek under rain fed and mid hill conditions of Himachal Pradesh. In *Indian Journal of Agricultural Research* Vol. 37, 2003, 1, p. 52-55.

SHARMA, S. 2001. Growth, physiological and yield aspects of mungbean (*Vigna radiata*) as affected by inoculation treatment by different strains of *Bradyrhizobium* culture. In *I. J. Res. Crop* Vol. 2, 2001, p. 112-115.

SOLIMAN, S. - GHANDOUR I.A. – ABBADY, K.A. 1996. Effect of nitrogen and phosphorus supply and of *Rhizobium* and VAM fungus inoculants on dinitrogen fixation in soybean. In *Folia Microbiological,* Vol. 41, 1996, 2, p.197- 200.

TARAFDAR, J.C. - RAO, A.V. 2001. Response of cluster bean to *Glonus mossea* and *Rhizobium* in an arid soil fertilized with nitrogen, phosphorus and farm yard manure. In *Journal of the Indian Society of Soil Science* Vol. 49, 2001, p. 751-755.

THAKUR, A.K. – PANWAR, J.D.S. 1995. Effect of *Rhizobium* -VAM interactions on growth and yield in mungbean (*Vigna radiata* L.) under field conditions. In *Indian J. Plant Pathol*. Vol. 38, 1995, p. 62-65.

VANCE, C.P. – GRAHAM, P.H. 1995. In Nitrogen Fixation: Fundamentals and Applications. IA Tikhonovich, et al. (eds.) Kluwer Academic Publishers, Dordrecht. 1995, 77-86 p.

VERMA, D.P.S. – FORTIN, M.G. – STANLEY, V.P. – MAURO, S. – PUROHIT, S. – MORRISON, N. 1986. In Nodulins and nodulin genes of *Glycine max*: A perspective. *Plant Mol. Biol.* Vol. 7, 1986, p. 51-61.

YANNI, Y.G. 1992. Performance of chickpea, lentil and lupine nodulated with indigenous or inoculated rhizobia micro-partners under nitrogen, boron, cobalt and molybdenum fertilization schedules. In *World J. Microbiol. Biotechnol.* Vol.8, 1992, p. 607- 613.

ZAR, J.H. 2003. Biostatistical analysis (Ed IV) Pearson Education Inc., New Delhi. 2003, p. 212-218.