P. J. Bot., 42(2): 863-871, 2010.

GROWTH, NODULATION AND YIELD RESPONSE OF SOYBEAN TO BIOFERTILIZERS AND ORGANIC MANURES

ARSHAD JAVAID* AND NASIR MAHMOOD

Institute of Mycology and Plant Pathology,
University of the Punjab, Quaid-e-Azam Campus Lahore, Pakistan
**E-mail: arshadjpk@yahoo.com*

Abstract

A field experiment was conducted to investigate the effect of a symbiotic nitrogen fixing bacterium *Bradyrhizobium japonicum* strain TAL-102 and a commercial biofertlizer EM (effective microorganisms) on growth, nodulation and yield of soybean [*Glycine max* (L.) Wilczek] in soils amended either with farmyard manure or *Trifolium alexandrinum* L. green manure @ 20 tons ha^{-1} each. In green manure amendment, *B. japonicum* inoculation significantly enhanced number and biomass of nodules resulting in a significant increase of 27, 65 and 55% in shoot biomass and number and biomass of pods, respectively. In farmyard manure amended soil, *B. japonicum* inoculation significantly enhanced fresh biomass of nodules. As a result a significant increase of 45 and 47% in shoot biomass and number of pods was recorded, respectively. Generally, the effect of sole EM application on various studied parameters was insignificant in both the soil amendment systems. Combined application of EM and *B. japonicum* in green manure amended soil reduced shoot growth and number of pods as compared to sole *B. japonicum* inoculation. Conversely, in farmyard manure amendment, plants co-inoculated with *B. japonicum* and EM exhibited highest and significantly greater shoot biomass, and number and biomass of pods as compared to all other treatments. The present study concludes that soybean yield can be significantly enhanced by the application of *B. japonicum* and EM in farmyard manure amendment.

Introduction

Soybean [*Glycine max* (L.) Wilczek] is a globally important oilseed crop and source of high quality protein for human consumption, used as fodder for animal and is also important in improved crop rotation systems (Manyong *et al*., 1996; Carsky *et al*., 1997). When in symbiotic association with *Bradyrhizobium japonicum*, soybean plants can fix up to 200 kg N ha^{-1} yr^{-1} (Smith & Hume, 1987), reducing the need for expensive and environmentally damaging nitrogen fertilizer. Nodulation of soybean requires specific *Bradyrhizobium* species (Abaidoo *et al.*, 2007). In soils where the soybean crop has not been grown previously, compatible populations of bradyrhizobia are seldom available (Abaidoo *et al*., 2006). The nitrogen demand of soybean can be supplied *via* biological nitrogen fixation through the inoculation with selected *Bradyrhizobium japonicum*/*B. elkanii* strains. Biological nitrogen fixation can reduced the need for N fertilizers, resulting in an economy estimated in US$ 3 billion per crop season (Nicolás *et al.*, 2006). The symbiosis between soybean and bradyrhizobia results from a complex process involving many genes of both partners that leads to the formation of N_2-fixing nodules in roots (Provorov & Vorob'ev, 2000).

EM (Effective Microorganisms) Technology of nature farming was introduced by Japanese scientists in late 1980s (Higa, 1989). EM culture consists of co-existing beneficial microorganisms, the main being the species of photosynthetic bacteria viz., *Rhodopseudomonas plastris* and *Rhodobacter sphacrodes*; lactobacilli viz., *Lactobacillus plantarum*, *L. casei* and *Streptococcus lactis*; yeasts (*Saccharomyces* spp), and actinomycetes (*Strptomyces* spp.). These microorganisms improve crop growth and yield by increasing photosynthesis, producing bioactive substances such as hormones and enzymes, controlling soil diseases and accelerating decomposition of lignin materials in the soil (Higa, 2000; Hussain *et al.,* 2002). Application of EM is known to enhance crop growth and yield in many crops both leguminous and non-leguminous (Sheng & Lian, 2002; Javaid, 2006, 2009; Khaliq *et al.,* 2006; Daiss *et al.,* 2008). In Pakistan, this technology of nature farming was introduced in 1990 by the Nature Farming Research Centre, University of Agriculture, Faisalabad. Numerous field and green house trials are indicative of the benefits of this technology for crop production, as a probiotic in poultry and livestock rations, and to enhance the composting and recycling of municipal/industrial wastes and effluents (Hussain *et al.,* 1999). At present Nature Farming Research and Development Foundation is disseminating this technology throughout the country. Three commercial products viz. EM Bioaab, EM Biovet and EM Biocontrol have been introduced to the farmers by this organization. EM Bioab is used in agricultural crops along with organic manures as a substitute of chemical fertilizers. EM Biovet is used in livestock and poultry production while EM Biocontrol is used in crops, vegetables and orchards for prevention and remedy of diseases and insect pest attack (Hussain *et al.,* 2002).

Soybean [*Glycine max* (L.) Wilczek] is a comparatively new leguminous oil-seed crop for the plains of Pakistan. The objective of this study was to evaluate the effect of *Bradyrhizobium japonicum* st. TAL-102 inoculation and EM application on plant growth, nodulation and yield of soybean in soils amended with farmyard and green manures.

Materials and Methods

Soil and environmental characteristics: A field experiment was conducted in Botanical Garden, University of the Punjab, Lahore, Pakistan during August-October 2004. The soil of experimental area was loamy textured having organic matter 0.9%, pH 8.2, nitrogen 0.05%, available phosphorus 14 mg kg^{-1} and available potassium 210 mg kg^{-1} of soil. The city of Lahore is located on latitude 31.57 N and longitude 74.31 E. The average rainfall during August, September and October in the area was 13.25, 6.25 and 1.0 mm, mean maximum temperature 92, 92 and 88 ˚F and mean minimum temperature 82, 79 and 69 ˚F, respectively. The day duration during August, September and October ranged from 12 h 52 min., to 13 h 41 min., 11 h 54 min., to 12 h 51 min., and 10 h 57 min., to 11 h 52 min., respectively. The weather was mostly clear with bright sunshine during the experimental period.

Soil amendments: Fresh farmyard manure @ 20 t ha^{-1} was thoroughly mixed in the field plots. Similarly *Trifolium alexandrinum* green manure, grown in the respective plots during December 2003, was thoroughly mixed in the soil @ 20 t ha^{-1} during February 2004 (Hussain *et al.,* 1998). The plots were irrigated with tap water. Plots were left for 30 days to decompose the manures and irrigated whenever required to maintain the soil moisture. In March 2004, mungbean [*Vigna radiata* (L.) Wilczek] was sown in these plots. After harvesting of mungbean, soybean was planted in the same fields in August 2004 for the present study.

EM application: Effective microorganisms' cultures under the commercial name of EM Bioaab were obtained from Nature Farming Research and Development Foundation Faisalabad, Pakistan. The stock culture was diluted to prepare 0.2% solution by adding tap water. The fresh solution was used immediately after preparation. The respective EM treated plots received dilute EM solution @ 2 L m^{-2} throughout the experimental period at fortnight intervals.

***Bradyrhizobium japonicum* inoculation:** Seeds of soybean with uniform shape, size and weight were surface sterilized with 1% sodium hypochlorite solution for 10 minuets followed by several washings with sterilized water. These surface sterilized seeds were pelted with peat based single strain inoculum of *B. japonicum* st. TAL-102 with concentrated sugar solution as an adhesive. Six seeds of soybean were sown in each row with 18 cm interplant spacing.

Experimental design and treatments: A combination of organic manures, EM and *B. japonicum* was arranged in a split-split plot design with organic manures as main plots, EM application as subplots and *B. japonicum* as sub-subplots. Each sub-subplot measured 1.5×1 m^2. There were four treatments for each of the two soil amendment systems. These were i). control, ii). EM application, iii). *B. japonicum* inoculation and iv). EM + *B. japonicum*. Each treatment was replicated thrice.

Harvesting schedule: Six replicate plants of each treatment were uprooted from the centre of the plots at maturity. Nodules were separated from roots and counted. The roots, shoots, nodules and pods were dried in oven at 70 °C to constant weight and weighed. Data were averaged on per plant bases.

Statistical analysis: Data regarding various root and shoot growth, nodulation and yield parameters were subjected to analysis of variance (ANOVA) followed by Duncan's Multiple Range Test to delineate mean differences (Steel & Torrie, 1980).

Results and Discussion

Effect of soil amendments: Effect of soil amendments was significant for shoot length, number and biomass of nodules, and pod number. The values of these parameters were generally higher in farmyard manure than in green manure amendment. Soil amendments exhibited an insignificant effect on root, shoot and pod biomass (Table 1, Figs. 1-3). Earlier Javaid *et al*., (2008) reported better growth and yield of wheat in farmyard manure than in green manure amended soil. It could be attributed to different mineralization rates and nutrient availability in the two soil amendment systems at different growth stages of the plant (Kautz *et al*., 2006).

Effect *B. japonicum* inoculation: Analysis of variance indicated significant effect of *B. japonicum* inoculation on all the studied parameters of plant growth, nodulation and yield (Table 1). In green manure amendment, *B. japonicum* inoculation significantly enhanced number, and fresh and dry biomass of nodules by 25, 35 and 20%, respectively. Consequently, a significant increase of 27, 65 and 55% was observed in shoot biomass, and number and biomass of pods, respectively. In contrast, the effect of *B. japonicum* inoculation on root biomass was insignificant in this soil amendment system (Figs. 1-3). In farmyard manure, *B. japonicum* inoculation failed to increase the number of nodules. However, generally nodules size in inoculated treatment was greater than in control. Consequently, an increase of 42 and 19% in fresh and dry biomass of nodules, respectively, was recorded in inoculated plants. As a result, a significantly greater shoot length, shoot biomass, root biomass, and number of pods were recorded in inoculated plants as compared to uninoculated control. *B. japonicum* inoculation failed to enhance pod biomass significantly in this soil amendment system. Increase in plant growth, nodulation and yield of soybean by *B. japonicum* strains have also been reported in other countries like Canada (Mabood *et al.,* 2005), South Africa (Botha *et al.,* 2004) and Brazil (Hungria *et al.,* 2001). Recently, Javaid *et al*., (2006) have reported that *B. japonicum* st. TAL-102 is also effective in increasing nodulation, plant growth and yield in black gram [*Vigna mungo* (L.) Hepper].

Table 1. Analysis of variance for various growth, yield and nodulation characters of soybean as affected by soil amendments and effective microorganisms application at two growth stages.

Trait	df	Mean squares							
		Shoot length	Shoot dry wt.	Root dry wt.	No. of nodules	Nodules fresh wt.	Nodules dry wt.	No. of fruits	Fruit dry wt.
		333***	25***	0.12*	38***	0.27***	0.12*	212***	38***
Amendments (A)	1	154**	4.3 ns	0.01 ns	133***	0.90***	0.01 ns	200***	9.2 ns
EM	1	8 ns	3.4 ns	0.25*	4 ns	0.12*	0.24*	24 ns	15*
Bradyrhizobium (B)	1	728***	109***	0.40**	61**	0.73***	0.41**	900***	155***
A×EM	1	420***	45***	0.04 ns	30*	0.03 ns	0.04 ns	200***	33**
A×B	1	12 ns	12 ns	0.11 ns	37*	0.01 ns	0.11 ns	0.08 ns	0.01 ns
EM×B	1	0.3 ns	2.4 ns	0.04 ns	1.3 ns	0.03 ns	0.04 ns	0.75 ns	16*
A×EM×B	1	21 ns	1.5 ns	0.01 ns	3 ns	0.07 ns	0.01 ns	37 ns	37**
Error	40	19	3.6	0.049	7.15	0.027	0.049	12	3.8
Total	**48**								

*, **, ***, Significant at $p \leq 0.05$, 0.01 and 0.001, respectively.

ns: Non-significant

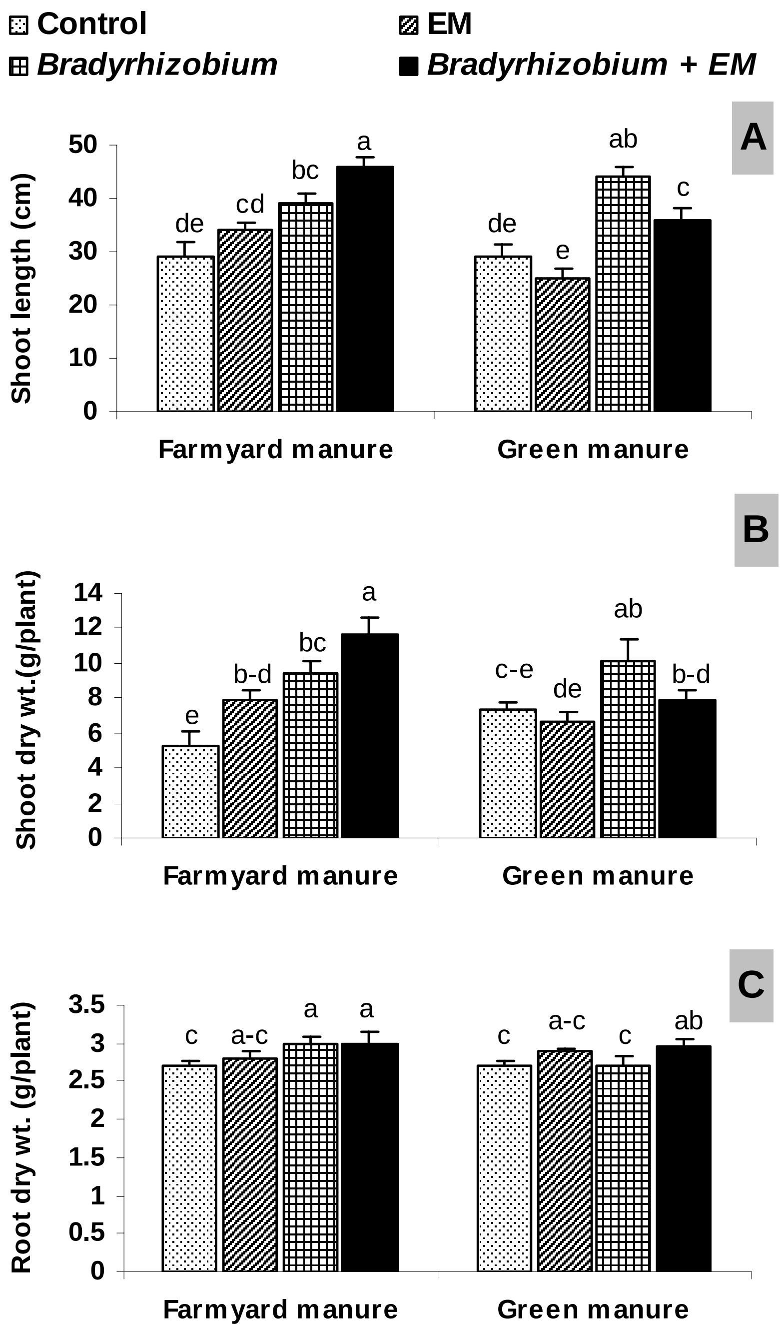

Fig. 1. Effect of soil amendments, *Bradyrhizobium japonicum* and EM application on shoot and root growth in soybean. Vertical bars show standard error of means of six replicate plants. Values with different letters show significant difference ($p \leq 0.05$) as determined by Duncan's Multiple Range Test.

Effect of EM application: EM application significantly enhanced shoot biomass in farmyard manure. In contrast to that effect of EM application on all other studied plant growth, nodulation and yield parameters was insignificant in either of the two soil amendment systems (Figs. 1-3). These results are in agreement with the findings of earlier workers who found that the effect of soil application of effective microorganisms on crop growth and yield was usually not evident or even negative particularly in the first test crop (Bajwa *et al.,* 1999; Xu, 2000; Javaid, 2006) possibly because introduced effective microorganisms have to face a competition with soil indigenous microflora (Bajwa *et al.,* 1995). Generally crop growth and yield with effective microorganisms application tends to increase gradually as subsequent crops are grown (Javaid *et al.*, 2000a,b). According to Kinjo *et al.*, (2000) the lack of consistency in results of the experiments regarding effective microorganisms application may be due to variable cultural conditions employed in previous studies. Imai & Higa (1994) stated that the observed decline in crop yields can often be attributed to the fact that soils, where conventional farming is practiced, have become disease-inducing or putrefactive soils from long-term use of pesticides and chemical fertilizers. Consequently, it takes time to establish a disease-suppressive or zymogenic soil. Until this conversion process is completed, it is virtually impossible to exceed crop yields that were obtained with conventional farming methods.

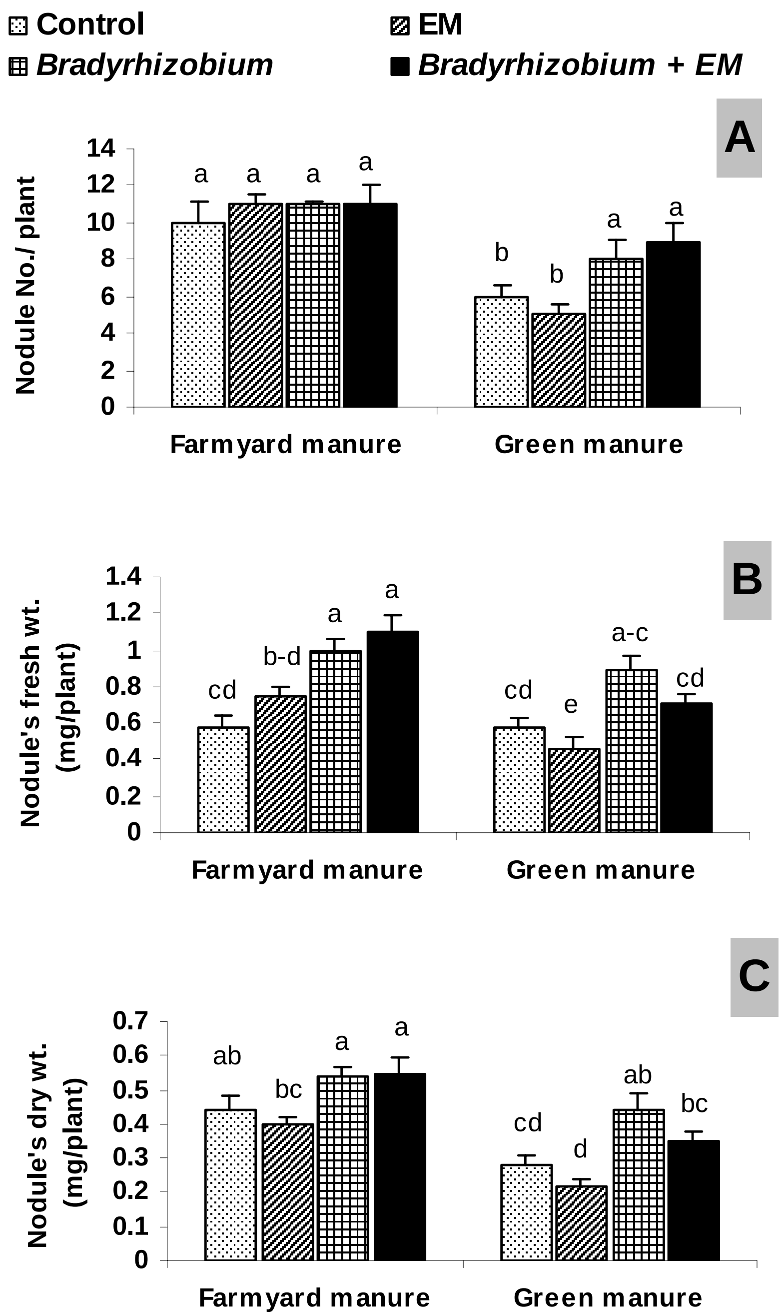

Fig. 2. Effect of soil amendments, *Bradyrhizobium japonicum* and EM application on nodulation in soybean. Vertical bars show standard error of means of six replicate plants. Values with different letters show significant difference (p≤0.05) as determined by Duncan's Multiple Range Test.

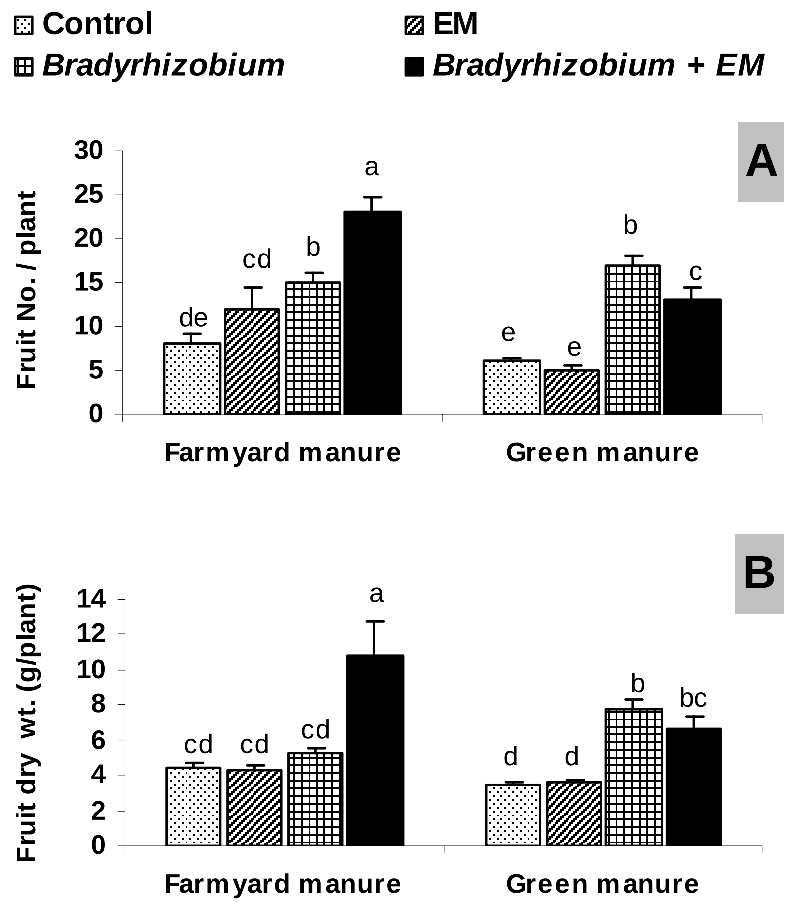

Fig. 3. Effect of soil amendments, *Bradyrhizobium japonicum* and EM application on number and biomass of fruits in soybean. Vertical bars show standard error of means of six replicate plants. Values with different letters show significant difference ($p \leq 0.05$) as determined by Duncan's Multiple Range Test.

In green manure amendment affect of combined inoculation of *B. japonicum* and EM was insignificant on all the studied parameters as compared to sole inoculation of *B. japonicum.* However, in farmyard manure amendment co-inoculated plants exhibited significantly greater shoot biomass, pod number and pod biomass as compared to control and plants inoculated with either alone. There was 55, 32 and 20% increase in shoot biomass in combined inoculated plants over control, and separate inoculations of EM and *B. japonicum,* respectively. Similarly, the increase in pod number 65, 48 and 35% and increase in pod biomass was 58, 60 and 52% in combined inoculated plants over control and separate inoculations of EM and *B. japonicum,* respectively. Effect of EM application on number and biomass of nodules in *B. japonicum* inoculated plants was insignificant in both the soil amendment systems (Figs. 1-3).

Conclusion

The present study indicates that the benefits of biofertilizers can be best exploited if they are applied with right combination of soil amendments. Both green manure and farmyard manure amendments were suitable to achieve better crop growth, nodulation and yield by *B. japonicum* inoculation. The efficacy of *B. japonicum* in increasing crop growth and yield can be further enhanced by EM application in farmyard manure amended soil.

Acknowledgement

Prof. Dr Tahir Hussain, Director Nature Farming Research and Development Centre, Faisalabad, Pakistan provided us with cultures of effective microorganisms, and Dr. Fouzia Yousaf Hafeez, National Institute for Biotechnology and Genetic Engineering, Faisalabad, Pakistan provided the *B. japonicum* inoculum that is gratefully acknowledged.

References

Abaidoo, R.C., H.H. Keyser, P.W. Singleton, K.E. Dashiell and N. Sanginga. 2006. Population size, distribution, and symbiotic characteristics of indigenous *Bradyrhizobium* spp., that nodulate TGx soybean genotypes in Africa. *App. Soil Ecol.,* 34: 57-67.

Abaidoo, R.C., H.H. Keyser, P.W. Singleton, K.E. Dashiell and N. Sanginga. 2007. Population size, distribution, and symbiotic characteristics of indigenous *Bradyrhizobium* spp. that nodulate TGx soybean genotypes in Africa. *App. Soil Ecol.,* 35: 57-67.

Bajwa, R., A. Javaid and N. Rabbani. 1999. EM and VAM technology in Pakistan. VII: Effect of organic amendments and effective microorganisms (EM) on VA mycorrhiza, nodulation and crop growth in *Trifolium alexandrianum* L. *Pak. J. Biol. Sci.,* 2: 590-593.

Bajwa, R., A. Javaid and Z. Tasneem. 1995. Response of indigenous soil microflora to EM inoculation in Pakistan. *Biota,* 1: 73-79.

Botha, W.J., J.B. Jaftha, J.F. Bloem, J.H. Habig and I.J. Law. 2004. Effect of soil bradyrhizobia on the success of soybean inoculant strain CB 1809. *Microbiol. Res.,* 159: 219-231.

Carsky, R.J., R. Abiadoo, K.E. Dashiell and N. Sanginga. 1997. Effect of soybean on subsequent maize grain yield in Guinea savannah of West Africa. *Afr. Crop Sci. J.*, 5: 31-39.

Daiss, N., M.G. Lobo and R. Socorro, U. Bruckner, J. Heller and M. Gonzaler. 2008. The effect of three organic pre-harvest treatments on Swiss chard (*Beta vulgaris* L. var. *cycla* L.) quality. *Eur. Food Res. Technol.,* 226: 345-353.

Higa, T. 1989. Studies on application of microorganisms in nature farming. II: The practical application of effective microorganisms (EM). In: *Proceedings of 7th IFOAM Conference, Ouagadougou, Burkina Faso, West Africa*.

Higa, T. 2000. What is EM technology? *EM World J.,* 1: 1-6.

Hungria, M., R.J. Campo, L.M.O. Chueire and Grange M. Megias. 2001. Symbiotic effectiveness of fast-growing rhizobial strains isolated from soybean nodules in Brazil. *Biol. Fertil. Soils,* 33: 387-394.

Hussain T., G. Jilani, T. Javaid and S.H. Tahir. 1998. Nature farming with EM technology for sustainable crop production in Pakistan. In: 4th *Proc. Intl. Conf. Kysei Nature Farmingheld in Paris, France*, June 19-21, 1995. pp. 71-78.

Hussain, T, T. Javaid, J.F. Parr, G. Jilani and M.A. Haq. 1999. Rice and wheat production in Pakistan with effective microorganisms. *Am. J. Alter. Agric.,* 14: 30-36.

Hussain, T., A.D. Anjum and J. Tahir. 2002. Technology of beneficial microorganisms. *Nature Farming Environ.,* 3: 1-14.

Imai, S. and T. Higa. 1994. Kyusei Nature Farming in Japan. Effect of EM on growth and yield of spinach. In: *Proc. 2nd Inter. Conf. Kyusei Nature Farming, Brazil*, Oct.7-11, 1991. pp. 92-96.

Javaid, A. 2006. Foliar application of effective microorganisms on peaas an alternative fertilizer. *Agron. Sustain. Dev.,* 26: 257-262.

Javaid, A. 2009. Growth, nodulation and yield of black gram [*Vigna mungo* (L.) Hepper] as influenced by biofertilizers and soil amendments. *Afr. J. Biotechnol.,* 8: 5711-5717.

Javaid, A., R. Bajwa and T. Anjum. 2006. Response of black gram to *Bradyrhizobium japonicum*inoculation under different soil amendment systems. *Pak. J. Bot.,* 38: 851-857.

Javaid, A., R. Bajwa and T. Anjum. 2008. Effect of heat sterilization and EM (effective microorganisms) application of wheat (*Triticum aestivum* L.) grown in organic matter amended soils. *Cereal Res. Comm.,* 36(3): 489-499.

Javaid, A., R. Bajwa, I. Siddiqi and U. Bashir 2000a. EM and VAM Technology in Pakistan VIII: Nodulation, yield and VAM colonization in *Vigna mungo* in soils with different histories of EM application. *Int. J. Biol. Agric.,* 2: 1-5.

Javaid, A., R. Bajwa, N. Rabbani and M. Uzma. 2000b. EM and VAM Technology in Pakistan. IX: Effect of EM application on growth, yield, nodulation and VA mycorrhizal colonization in *Vigna radiata* (L) Wilczek. *Pak. J. Biol. Sci.,* 3: 694-698.

Kautz, T., C. López-Fando and F. Ellmer. 2006. Abundance and biodiversity of soil microarthropods as influenced by different types of organic manure in a long-term field experiment in Central Spain. *App. Soil Ecol.,* 33: 278-285.

Khaliq, A., M.K. Abbasi and T. Hussain. 2006. Effect of integerated use of organic and inorganic nutrient sources with effective microorganisms (EM) on seed cotton yield in Pakistan. *Bioresour. Technol.,* 97: 967-972.

Kinjo, T., K. Perez, E. de Almeida, M.A.G. Ramos and J.O. de Oliveia. 2000. Plant growth affected by EM-Bokashi and chemical fertilizers. *Nature Farm. Environ.,* 1: 33-38.

Mabood, F., X. Zhou, K.D. Lee and D.L. Smith. 2005. Methyl jasmonate, alone or in combination with genistein, and *Bradyrhizobium japonicum* increases soybean (*Glycine max* L.) plant dry matter production and grain yield under short season conditions. *Field Crops Res.,* 95: 412-419.

Manyong, V.M., K.E. Dashiell, B. Oyewole and G. Blahut, 1996. Spread of new Soybean varieties in a traditional soybean growing area of Nigeria. A Seminar Paper in the Second Symposium of the African Association of Farming Systems Research Extension and Training (AAFSRET), 20–23 August 1996, Ouagadougou, Burkina Faso pp. 151-162.

Nicolas, M.F., M. Hungria and C.A.A. Arias. 2006. Identification of quantitative trait loci controlling nodulation and shoot mass in progenies from two Brazilian soybean cultivars. *Field Crops Res*., 95: 355-366.

Provorov, N.A. and N.I. Vorob'ev. 2000. Evolutionary genetics of nodule bacteria: molecular and populational aspects, *Russ. J. Genet.,* 36: 1323-1335.

Sheng, Y.P. and X.H. Lian. 2002. Influence of EM Bokashi on nodulation, physiological characters and yield of peanut in nature farming fields. *J. Sustain. Agric.,* 19: 105-112.

Smith, D.L. and D.J. Hume. 1987. Comparison of assay methods for N_2 fixation utilizing white bean and soybean. *Can. J. Plant Sci.,* 67: 11-19.

Steel, R.G.D. and J.H. Torrie. 1980. *Principles and Procedures of Statistics.* McGraw Hill Book Co., Inc, New York, USA.

Xu, H.L. 2000. Effects of a microbial inoculant and organic fertilizers on the growth, photosynthesis and yield of sweet corn. *J. Crop Prod.,* 3: 183-214.

(Received for publication 13 February 2009)