Pak. J. Bot. 33 (special issue) 2001

GROWTH, NODULATION AND VAM COLONIZATION RESPONSE OF *VIGNA RADIATA* (L.) WILCZEK TO CO-INOCULATION OF BIOPOWER AND EFFECTIVE MICROORGANISMS (EM)

RUKHSANA BAJWA, NASIR MAHMOOD AND ARSHAD JAVAID

Departmet of Botany, University of the Punjab, Quaid-e-Azam Campus, Lahore. Pakistan.

Abstract

A pot experiment was conducted to study response of plant growth, nodulation and vesicular arbuscular mycorrhizal (VAM) colonization to co-inoculation of Biopower (BP) and Effective Microorganisms (EM) when *Vigna radiata* (L) Wilczek was grown in farmyard manure (FYM) and *Trifolium* green manure (GM) amended soils. Maximum VAM colonization, nodulation and growth were achieved in co-inoculation treatments of BP and EM in the presence of FYM + GM or FYM alone. BP enhanced yield in the presence of organic amendments. Nitrogen content of shoot was significantly increased by co-inoculation of BP and EM in the presence of FYM. VAM colonization was stimulated by co-inoculation of EM and BP in the presence of organic amendments.

Introduction

Imbalance and frequent use of agrochemicals like fertilizers and pesticides not only pollute the environment to a great extent but also food produced under such farm management may not be safe or of good quality. Therefore, in the recent past some successful efforts have been made to at least partially substitute chemical with biological methods. One such efforts has been made by Higa (1986) by introducing EM culture that consists of 80 species of co-existing microorganisms such as photosynthetic and nitrogen fixing bacteria, yeast and lactobacilli which improve crop yield by increasing photosynthesis, nitrogen fixation, controlling diseases and accelerating decomposition of lignin materials in the soil (Hussain *et al.*, 1994). The studies of EM application in peanut and carrot (Arakawa, 1991), corn (Punchaban, 1991), sweet potato (Sangakkara, 1994), mungbean (Sangakkara and Higa, 1994)and rice (Minami and Higa, 1994) have reported an increase in crop growth and yield. Sanagakkara and Higa (1994) observed increased nodulation and nitrogen fixation in legumes due to EM application. Very encouraging results of increase in crop growth and yield by the application of EM have also been observed in wheat, rice, pea and sunflower in Pakistan (Rashid *et al.*, 1993; Hussain *et al.*, 1993; Javaid *et al.*, 1995, 1997, 1999, 2000).

Legumes are unique among crop plants in that they are capable of contributing a limiting resource to the agroecosystem by fixing N_2. Soil bacteria belonging to the family Rhizobiacae and falling in the genera *Rhizobium* and *Bradyrhizobium* are capable of forming nodules on legume plants. While colonizing the nodules, the bacteria develop into N_2-fixing bacteroids providing the host plant with NH_4^+ as nitrogen source. In return, the plant supplies the bacteroids with vital organic compounds. During the establishment of active symbiosis, a well coordinate exchange of molecular signaling between the legume host and bacterial partner occurs leading ultimately to the formation of nodules (Lakshminarayana and Sharma, 1994). The scientists of Nuclear Institute for Biotechnology and Genetic Engineering, Faisalabad isolated a number of bacterial strains belonging to the

genera *Rhizobium* and *Bradyrhizobium*. After testing in laboratory and studying performance in the field, efficient bacterial strains were selected for Biopower production. A suitable carrier material is being used to ensure maximum survival of the *Rhizobium/Bradyrhizobium* during storage and transportation.

Mungbean [*Vigna radiatu* (L.) Wilczek] is a major summer pulse crop and constitutes an important source of readily available proteins in the cereal-based diet of common man in Pakistan. It is consumed as dry beans. It is also used as fodder for livestock & often incorporated in soil for increasing soil fertility. In Pakistan mungbean is grown on an area of 192400 ha with an annual production of 89500 tons with an average seed yield of 465kg/ha (Anonymous, 1997). The present study was designed to evaluate the effect of co-inoculation of EM and Biopower on growth, yield, N nutrition and VAM colonization in *Vigna radiata* in the presence and absence of soil organic amendments.

Materials and methods

Experiment was conducted in earthen pots of 24-cm diameter, each containing 5 Kg of sandy loam soil. Pot soil was either left un-amended or amended with farmyard manure (FYM) @ 10 g / 100 g soil, green manure (GM) @ 10 g / 100 g soil, or a mixture of FYM and GM @ 5+5 g / 100 g soil. Effect of Biopower and EM application on growth, nodulation, yield and VAM colonization in *V. radiata* was studied in the soils with and without organic amendments by employing the following treatments regimes.

1. Control (Without organic amendment and microbial inoculation)
2. EM
3. Biopower (BP)
4. BP + EM
5. BP + FYM
6. BP + FYM + EM
7. BP + GM
8. BP + GM + EM
9. BP + FYM + GM
10. BP + FYM + GM + EM

Six surfaces sterilized pre-soaked seeds with or without Biopower dressing were sown in respective pots which were thinned to three uniform seedlings after germination. Each treatment was replicated three times. Pots were irrigated with tap water. The respective EM treatments received a dilute suspension of EM (1:500) once a week. Plants were harvested at 30, 50 and 70 days after sowing. At each harvest plants were carefully uprooted and washed thoroughly. Nodules were separated from roots and counted. Fresh dry weights of root, shoot and nodules were recorded. At final harvest pod number and dry weight was also recorded.

A sub-sample of fresh roots was cleared and stained by procedure of Philips and Hayman (1970). Extent of mycorrhizal infection was estimated by Slide Length Method (Giovenetti and Mosse 1980). Arbuscular and vesicular infection were quantified by counting these structures per cm of root length. Shoot nitrogen content was determined by Kjeldahs method (Bremner, 1965). All the data were analyzed by applying Duncan's Multiple Range Test (Steel and Torrie, 1980)

Results

Effect of EM and Biopower on shoot growth in *V. radiata*: Application of EM and Biopower significantly reduced the shoot length 30 days after sowing (DAS) in soil without any organic amendment. However, at later growth stages viz. 50 and 70 DAS there was not any significant difference in shoot length between control and EM or Biopower treated plants. Co-inoculation of EM and Biopower persistently reduced shoot length at all the growth stages in soil with out organic amendments (Table-1). Shoot length was significantly increased as compared to control when Biopower was applied, either alone or in combination with EM, along with FYM or GM (Table-1).

Shoot fresh and dry biomass was reduced due to Biopower and EM in the absence of organic manures, 30 and 50 DAS. However 70 DAS there was a significant increased in shoot biomass due to EM application (Table-1). In soils amended either with FYM or mixture of FYM and GM, there was a significant increased in shoot biomass due to Biopower and EM as compared to control. In GM amended soil effect of Biopower and EM application was less pronounced than in FM amended soil (Table-1).

Effect of EM and Biopower on root growth in *V. radiata*: Root length was invariably reduced in all the treatment at 30 days growth stage as compared with un-inoculated control. However, at later growth stage root length response to different treatments was different, in soil without any organic amendment root length was increased by EM application similarly in FYM amended soil, Biopower application either alone or in combination with EM, enhanced root length, Biopower and EM application in GM amended soil adversely affected the root length. Effect was significant at all the three growth stages (Table-1).

Effect of EM and Biopower on nodulation in *V. radiata*: EM application. in un-amended soil decreased the nodule number at initial growth stage. Biopower inoculation either alone or in combination with EM significantly enhanced nodule number as compared to un-inoculated control (Table-2). Biopower application in FMM amended soil had no marked effect on nodule number at early growth stage. However, maximum number of nodule per plant were recorded when Biopower inoculation was done in soil amended with a mixture o FYM and GM. EM application to this treatment reduced the nodules number (Table-2). Biopower application either alone or in combination with EM significantly reduced nodule number in GM amended soil at early growth stage (Table-2). The response of nodule fresh and dry weight to different treatment s was similar to that of nodule number (Table-2).

Effect of EM and Biopower on yield in *V. radiata*: In unattended soil the effect of Biopower and EM application on fruit number was insignificant. In FYM and FYM + GM amended soil Biopower application significantly enhanced fruit number EM application further enhanced the fruit number. In GM amended soil Biopower increased fruit number, however EM application suppressed the effect of Biopower (Fig. 1A). In FYM and FYM + GM amended soil, the effect of Biopower and EM application on fruit biomass was similar to that of fruit number. In GM amended

soils Biopower alone had an insignificant effect while co-inoculation of Biopower and EM significantly enhanced fruit biomass (Fig. 1B).

Effect of EM and Biopower on shoot nitrogen content in *V. radiata*: Separate inoculation of Biopwer and EM in unattended soil significantly increased the shoot N content. However, co-inoculation of EM and Biopower had insignificant effect on N content (Fig. 2). Biopower application in FYM and FYM +GM amended. Soils had a marked and significant effect on shoot N content that was further enhanced by EM application. EM and Biopower applications in GM amended soil significantly increased N content as compared to un-inoculated control. However the effect was less pronounced and significantly lower as compared to significantly lower as compared to FYM and FYM + GM amended soils (Fig. 2).

Effect of EM and Biopower on VAM colonization in *V. radiata*: Biopower application significantly enhanced extent of VAM colonization organic manure amended soils. EM application further supported the VAM colonization. Highest effect was observed in FYM and FYM + GM amended soil (Table -2). Arbuscular and vesicular infections also showed a similar response to Biopower and EM in organic manure amended soils. The response of arbuscular and vesicular infections to separate inoculation of EM and Biopower was also positive and significant however, less pronounced as compared to organic manure amended soils response of arbuscular and vesicular infections to co-inoculation in un-amended soil was significantly negative (Table-2).

Discussion

The results possessed from the present investigation revealed the superiority of Bio-power (BP) in combination with or without EM in the presence of FYM and mixed organic amendments (FYM + GM) in terms of growth and yield. EM application individually failed to support any promotive growth; however, in combined application with BP in the presence of organic amendments, a significant stimulus was obtained in the vegetative growth of the host.

Biopower an activated version of rhizobial inoculation considerably enhanced the yield of mycorrhizal legumes through increased nitrogen-fixation. This effect has recently been supported by numerous workers. Rajput (1994) has reported 13.57% greater yield of chickpea plants inoculated with this bio-culture of rhizobia. Similar results were observed by Thukral and Mishra (1993).A very significant response to induce inoculations of rhizobia in soils where indigenous populations were very low, have earlier been reported by several investigators (Singleton and Tavares, 1986; Thies *et al.*, 1991; Shah *et al.*, 1996). Stimulation in functionally more active nodulation and consequent increase in nitrogen-fixation as a result of appropriate *Rhizobium* inoculation is a time tested universal phenomenon and both earlier and recent investigations provide strong evidence that increased nodulation leads to increase nitrogen-fixation mediated through improved nitrogenase activity (Javaid *et al.*, 1994a ; Khan *et al.*, 2000) and thus yield as well as dry weight of the plant is highly increased (Herrera and Longeri, 1985).

Sole EM application has previously been shown to enhance both VAM colonization and nodulation with resultant stimulation in plant growth (Bajwa *et al.*, 1997). However, in present investigations EM in combination with BP, in soil

without any organic amendments displayed very adverse effects on plant growth. This can probably be attributed to development of competition among microorganisms for available nutrients. It is further evident that in the presence of organic amendments the co-inoculation of BP and EM provided beneficial enhancement in vegetative growth as well as biomass production. It is indicative of excessively enhanced decomposition of organic matter by Lactobacilli present in EM (Hussain *et al.,* 1994) and hence continuous release of nutrients to plant and consequently to associated mycorrhiza with functionally more balanced colonization (Tinker, 1978). The green manure as sole source of fertilization either with BP or in co-inoculation with BP & EM could not provide the desired benefits in terms of plant growth.The suppression of mycorrhizal association in soils amended with green manure has also been reported recently by Javaid *et al.* (2000) and the authors have attributed this effect to higher concentrations of nitrogen in leguminous green manuring crops.

It is evident from the present investigations that the coinoculation of BP and EM in FYM amended soil provided the maximum stimulus in mycorrhizal development as well as nodulation which enhanced the functioning of both associated microbes and host plant so resulted in desired yield response. FYM due to its low nitrogen content (Donahue *et al.,* 1983) favoured VAM colonization. It has also been pointed out that organic matter in FYM is likely to affect the mycorrhizae through its influence on soil structure, nutrient mineralization and soil water holding capacity (Johnson and Pfleger, 1992). Herinikumar and Bagyaraj (1989) have earlier shown that application of FYM to corn, millet and sunflower increased VAM fungi. EM application further enhanced the VAM colonization in FYM amended soils. The increased VAM colonization also enhanced nitrogen-fixing activity of the nodules (Pacovsky *et al.*, 1986), which in turn leads to maximum yield.

The photosynthetic bacteria in EM solution synthesize vitamins by effectively utilizing sunlight and also synthesize glucose and amino acids, using secretions from the roots and substances produced when organic matter is decomposed (Hussain *et al.,* 1994). These substances could be responsible to promote the VAM colonization and growth of the plants. Plant hormones, produced by soil micro-organisms are also known to stimulate VAM development (Azcon *et al.,* 1978).

Generally in GM amended treatments of BP with and without EM, poor development of mycorrhiza fails to support nitrogen-fixing activity of *Rhizobium* . Thus, growth, yield and nitrogen content of shoot insignificantly reduced as compared to FYM amended treatments of BP with or without EM. The addition of both the bio-cultures (BP & EM) either with FYM or FYM + GM highly intensified the arbuscular infection. The activity of combined bio-cultures accelerated the decomposition and uptake process of organic amendments which although being rich sources, the gradual release of nutrients helps to facilitate the strong mycorrhizal development. This observation is in agreement with Mosse (1973), who proposed that arbuscular infection was greatly influenced by nutrient supply. Initially the number of arbuscules and arbuscular infection was very high but in later stage vesicular infection was found to enhance.

This study concludes that in the presence of FYM both the bio-cultures greatly enhanced the vegetative and reproductive growth directly by increasing the

decomposition rate of organic matter and the availability of nutrients to plants was sufficiently increased through mycorrhizal association. Further support indirectly with increased mycorrhizal colonization consequent stimulation in nodulation and nitrogen fixation ultimately lead to enhancement of nitrogen content of the shoot. In the absence of organic amendments co-inoculation of BP and EM adversely affected the growth in general, which in fact might have arised because of antagonistic competition for available resources of the host plant resulting in the loss of desired benefits likely to be attained otherwise.Therefore, the present study highly recommends that the bio-active microbial cultures may only be employed in soils amended continuously with FYM to achieve a maximum manipulation in plant growth and yield.

Table 1. Effect of EM and Biopower on root and shoot growth of *Vigna radiata.*

Treatments	Shoot			Root		
	Length (cm)	Fresh wt. (g)	Dry wt. (g)	Length (cm)	Fresh wt. (g)	Dry wt. (g)
30 Days after sowing						
Control	16 b	0.86 b	0.26 f	21.6 a	0.19 f	0.10 b
EM	13 c	0.68 c	0.17 h	20.9 a	0.2 e	0.10 b
BP	15 c	0.71 b	0.21 g	18.2 ab	0.21 e	0.10 b
BP + EM	12 d	0.44 e	0.12 i	14.4 d	0.16 g	0.07 c
BP + FYM	19 a	1.92 d	0.54 a	14.6 d	0.31 c	0.12 b
BP + FYM +EM	20 a	2.1 a	0.50 b	17.7 b	0.37 a	0.15 a
BP + GM	18 ab	1.2 ab	0.29 de	11.3 e	0.16 g	0.06 d
BP + GM + EM	16 b	1.1 ab	0.28 e	9.8 f	0.22 d	0.07 c
BP + FYM + GM	19 a	1.8 a	0.48 c	15.6 c	0.33 b	0.15 a
BP + FYM GM + EM	16 b	1.3 a	0.30 d	11.6 e	0.19 f	0.07 c
50 Days after sowing						
Control	23 c	2.53 cd	0.66 c	21.5 a	0.56 e	0.25 c
EM	23 d	2.24 d	0.50 e	26.0 a	0.5 f	0.18 e
BP	22 cd	2.42 cd	0.53 d	22.4 b	0.71 c	0.26 bc
BP + EM	19 e	1.21 e	0.30 f	13.2 d	0.39 g	0.17 ef
BP + FYM	28 a	4.14 b	1.0 a	25.9 a	0.53 ef	0.20 d
BP + FYM +EM	27 a	3.94 b	0.98 a	21.9 bc	0.9 a	0.36 a
BP + GM	24 bc	2.7 c	0.7 b	11.5 e	0.25 h	0.10 f
BP + GM + EM	26 bc	3.1 bc	0.83 bc	15.0 d	0.26 h	0.11 f
BP + FYM + GM	27 a	5.2 a	1.1 a	19.8 c	0.63 d	0.21 d
BP + FYM GM + EM	28 b	4.7 ab	1.1 a	19.0 c	0.79 b	0.27 b
70 Days after sowing						
Control	27 f	6.5 de	3.93 a	22.8 d	3.6 b	3.1 c
EM	29 h	8.1 cd	4.63 ab	27.5 b	3.8 ab	3.2 b
BP	27 g	6.5 de	3.83 b	21.7 e	4.0 a	3.2 b
BP + EM	24 i	6.9 d	3.92 b	21.4 e	3.9 ab	3.15 b
BP + FYM	30 a	11.0 ab	4.82 a	22.4 d	4.3 a	3.5 a
BP + FYM +EM	31 b	10.5 b	4.71 a	28.5 b	4.0 a	3.5 a
BP + GM	24 de	6.9 d	4.0 b	17.0 f	3.5 ab	2.9 cd
BP + GM + EM	28 e	8.5 c	4.04 a	16.6 f	4.1 bc	3.5 a
BP + FYM + GM	30 c	9.9 bc	4.33 ab	24.3 c	4.0 a	3.1 c
BP + FYM GM + EM	36 d	12 a	5.7 a	32.1 a	3.9 a	3.8 a

For each growth stage values followed by different letters in vertical columns show significant difference (P = 0.05) as determined by DMR Test.

EM: Effective Microorganisms
BP: Biopower
FYM: Farmyard manure
GM: Green manure

Table 2. Effect of EM and Biopower on nodulation and VAM colonization in *Vigna radiata.*

Treatments	Nodulation			VAM colonization		
	Number	Fresh wt. (mg)	Dry wt. (mg)	No. of arbuscules /cm	No. of vesicles/cm	Extent of infection (cm/100 cm)
30 Days after sowing						
Control	3 d	5.5 d	2.8 d	167 g	7 g	19 e
EM	2 e	3.0 e	1.6 e	184 e	11	23 de
BP	4 c	7.3 c	7.0 b	182 f	9 f	20 e
BP + EM	5 c	12.7 b	6.1 bc	110 h	6 h	17 f
BP + FYM	4 cd	14.3 a	8.0 a	210 c	14 d	29 cd
BP + FYM +EM	8 a	7.8 c	4.4 d	231 a	21 a	37 a
BP + GM	2 e	4.4 de	1.9 e	194 d	12 e	25 d
BP + GM + EM	2 f	4.5 d	2.2 d	198 d	13 d	27 d
BP + FYM + GM	7 b	9.9 bc	5.3 c	221 b	19 b	30 c
BP +FYM GM+ EM	3 d	5.5 d	3.0 d	212 c	17 c	35 b
50 Days after sowing						
Control	5 d	13.3 e	4.6 g	189 i	9 g	31 g
EM	5 cd	11.0 e	4.9 f	214 g	13 f	32 f
BP	8 bc	27.7 d	5.5 e	210 h	14 f	31 g
BP + EM	6 c	41.5 de	4.9 f	125 i	8 g	28 h
BP + FYM	10 ab	61.0 a	9.9 d	303 a	23 c	44 c
BP + FYM +EM	10 a	41.1 b	16.6 a	255 d	29 b	53 b
BP + GM	5 cd	12.3 ef	4.9 e	222 f	16 e	36 e
BP + GM + EM	5 d	13.3 e	5.9 e	237 e	19 d	40 d
BP + FYM + GM	11 a	33.3 c	15.6 b	278 b	31 a	45 c
BP+ FYM GM +EM	9 b	32.2 c	12.1 f	262 c	24 c	57 a
70 Days after sowing						
Control	6 c	8.34 c	457 ef	52 h	131 h	35 h
EM	8 ab	7.90 cd	567 cd	73 f	145 f	40 f
BP	5 d	1034 ab	678 bc	65 g	141 g	37 g
BP + EM	3 d	827 c	470 e	44 i	111 i	33 hi
BP + FYM	8 b	1140 a	799 b	87 c	163 d	63 c
BP + FYM +EM	9 a	1160 a	821 a	105 a	187 a	77 b
BP + GM	3 e	788 d	478 e	77 e	157 e	43 e
BP + GM + EM	7 c	969 b	527 d	83 d	161 d	60 d
BP + FYM + GM	14 a	1199 a	815 a	94 b	181 b	75 c
BP+FYM+GM+EM	9 b	977 b	571 cd	89 c	172 c	68 a

For each growth stage values followed by different letters in vertical columns show significant difference (P = 0.05) as determined by DMR Test.

EM: Effective Microorganisms
BP: Biopower
FYM: Farmyard manure
GM: Green manure

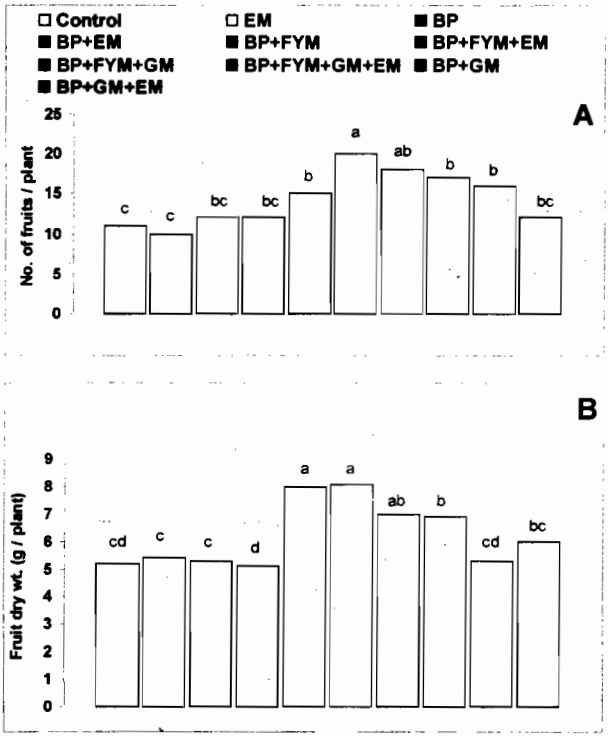

Figure 1. Effect of Biopower and EM on pod yield in *Vigna radiata* in farmyard manure (FYM) and green manure (GM) amended soils.
Bars with different letters at their tops show significantl different (P = 0.05).

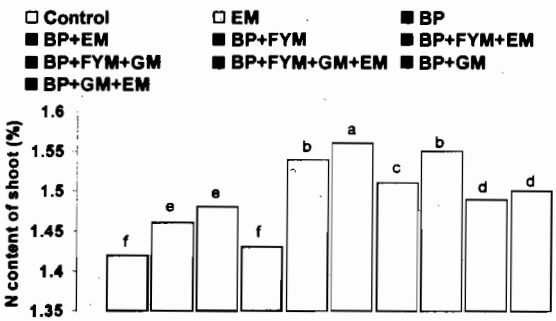

Figure 2. Effect of Biopower and EM on shoot N content in *Vigna radiata* in farmyard manure (FYM) and green manure (GM) amended soils.
Bars topped by the different letters are significantly different (P= 0.05).

Reference

Anonymous. 1997. Agriculture statistics of Pakistan, Ministry of food and Agriculture, Pakistan.

Arakawa, M.Y. 1991. The present state of Kyusei Nature Farming in Japan. *Proceedings of the first International conference on Kyusei Nature farming*, Khon Kaen University, Thailand. October, 17-21. pp 20-33.

Azcon, R., C. Azcon-Aguilar and J.M. Barea. 1978. Effect of plant hormones present in bacterial culture on the formation and responses of VA endomycorrhiza. *New Phytol.*, 80 : 359-364.

Bajwa, R., A. Javid, and T. Akhtar. 1997. EM and VAM Technology in Pakistan III: Effect of co-inoculation of EM and VAM on growth of wheat. *Sci. Int. (Lahore).*, 9: 311-314.

Bremner, J.M. 1965. Total nitrogen.Inorganic forms of nitrogen. In: Methods of soil analysis,(Eds)C.A.Black,D.D.Evans,J.L.White,L.E.Ensminge&F.E.Clark,*Am.Soc.Agron.*,Mad ison,Wisconsin.pp1149-1237.

Chenck. N.C and M.K. Kellam. 1978. The influence of vesicular arbuscular mycorrhizae on disease development. Florida *Agric. Exp. Stn. Tech. Bull.*, Florida.pp 798.

Donahue, R.L., R.W. Millder and J.C Sickluna. 1983. Soil. An introduction to soil & plant growth. Prentice. Hall, Inc., Englewood Clliffs., New Jersey.

Herinikumar, K.M & D.J. Bagyaraj. 1989. Effect of cropping sequence, fertilizers & farmyard munure on VAM fungi in differents crops over three consecutive seasons. *Biol. Fertil. Soil.*, 7: 173-175.

Herrera, A. and L. Longeri. 1985. Response of lentil (Lensculinaris Medik.) to inouclation with *Rhizobium leguminosarum. Cienciae Investigecian Agraria*,. 12 : 49-53.

Higa.T. 1986. Studies on the application of effective microorganisms in nature farming. *6th IFOAM conf.*, Aug. 18-21,1986, California University.

Hussain, T., G. Jilani, M. Yaseen, & M.A. Abbas. 1994. Effect of organic amendments & EM on crop production in Pakistan. *Proc. 2nd Inter. Conf. on Kyusei Nature forming*, brazil, Oct. 7-11, 1991. pp 132-139.

Hussain, T., G. Jilani, M. Yaseen and S.N. Akhtar. 1993. Prospects of Nature Farming with EM technology. *Proc. of the Ist National Seminar on Nature Farming*, June, 28, UAF, Pakistan, pp 41-49.

Javaid, A, I. Siddiqui, & R. Bajwa. 2000. EM & VAM technology in Pakistan X: effect of long term application of EM on growth, yield & VA mycorrhizal colonization in wheat. *Pak.. J. Phytopathol.*, 12: 26-30.

Javaid, A., R. Bajwa and I. Siddique. 1999. EM and VAM technology in Pakistan VI : Effect of EM (Effective Microorganisms) on VA mycorrhizal development and subsequent crop growth and yield in sunflower. *Pakistan Journal of Biological Sciences.*, 2(2) : 586-589.

Javaid, A., R. Bajwa, N. Rabbani and Q. Ahmad. 1995. Growth, nodulation, nitrogen and VAM colonization of Pea (*Pisum sativum* L.) in soil treated with EM. *Acta Scientia.*, 5(2): 1-6.

Javaid, A., R. Bajwa, Q. Ahmad and N. Rabbani. 1997. Effect of EM (Effective Microoganisms) application on growth, yield, nodulation and nitrogen nutrition in (*Pisum sativum* L.) in heat sterilized and unsterilized field soil. *Science International (Lahore)*, 9(3): 307-309.

Javaid, A.,S.H. Iqbal and F.Y.Hafeez.1994a.Effect of different strains of *Bradyrhizobium* and two types of VA mycorrhizae on nodulation in *Vigna radiata* Var NM 20-21, *Sci. Int..*, 6 (1) : 87-89.

Johnson, N.C and F.L. Pfleger.1992.. VA mycorrhizal & cultural stresses. In: Mycorrhizae in sustainable Agriculture, G.J., Bethlenfalvay and Linderman, R.G., (Eds):. American society of Agronomy.

Khan,G.H., I.A. Siddiqui and M.J. Zaki. 2000. Compatibility of Rhizobial strains with soybean (*Glycine max* L.). *Abstracts of 7th Natioanl Conference of plant scientists,* Nov. 14-16, P.U, Lahore. Pakistan. pp 58.

Lakshminarayana, K and P.K. Sharma. 1994. Molecular Biology of nodulation in legume-rhizobim symbiosis. In : Biology and Application of Nitrogen- Fixing organisms (Eds): A.B. Prasad and A. Vaishampayan, Scientific Publishers, Jodhpur. pp 155-172.

Minami, T. and T. Higa. 1994. Kyusei nature farming in Japan. Effect of EM on the yield of paddy rice. *Proc. 2nd inter. Cont. on Kyusei nature farming.*, Brazil, Oct.7-11, 1991, PP 97-102.

Mosse, B.1973.Advances in study of vesicular arbuscular mycorrhiza, *Ann. Rev. Phytopathology*, 11:171-196.

.Phillips, J.M. and D.S. Hayman. 1970. Improved procedure of clearing roots and staining parasitic and VA mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol, Soc.*, 5 : 158-161.

Punchaban, S. 1991. Effect of EM on growth and yield of corn. In : J.F. Parr, S.B. Hornick and C.E. Whitman (Eds). *Proc. Ist. Intl. conf. on Kyusei Natue Farming*. Oct.17-21, Khon Kaen, Thailand. Pub. USDA. Washington, D.C.

Rajput, A.L . 1994. Response of Chickpea to Rhizobium inoculation, date of sowing & phosphorous *Ind. J.Agron.*, 39: 584-587.

Rashid, A.,G. sidddiqe, S.M. Gill and M.Aslam. 1993. Contribution of microbes in crop production : Effect of a diazotropohic and a commercial EM4 inoculation on yield and N2 economy of wheat. In: T. Hussain, G.Jilani, R. Ahmad and S.Ahmad (Eds). *Proc. Ist. Nat. Sem. on Nature Farming*. June 28, 1993, Faisalabad, Pakistan.

Sangakkara, U.R . 1994. Effect of EM on the growth and yield of sweet potato in wet and dry seasons. *Proc. 2nd inter. Conf. on Kyusei Nature Farming*, Brazil, Oct. 7-11, 1991, pp 103-110.

Sangakkara, U.R. and T. Higa .1994. Effect of EM on nitrogen fixation by bush been and mungbeam. *Proc. 2nd Inter. Conf. on Kyusei Nature Farming*, Brazil, Oct. 7-11, 1991, pp 118-124.

Shah, N.H., F.Y. Hafeez, A. Hussain. K.A. Malik. 1996. Influence of Seasonal variation on the indigenous populationof *Rhizobium* in four Pakistani soils and competitive ability of the introduced rhizobia on field grown lentil. *Lens News letter*. 23: 32-37.

Singleton, P.W. and J.W. Tavares. 1986. Inoculation response of legumes in relation to number and effectiveness of indigenous *Rhizobium* population. *Applied and Environmental Microbiology*. 51: 1013-1018.

Steel, R.G.D. and J.H. Torrie. 1980. Principles and procedures of statistics. Mcgraw Hill Publishers. U.K. p 481.

Thies, J.E., P.W. Singleton and B.B. Bohlool. 1991. Influence of the size of indigenous rhizobial populations on establishment and symbitic performance of introduced *rhizobia* on field grown legumes. *Applied and Environmental Microbiology*. 57 : 19-28.

Thukral, V. and C.M. Mishra. 1993. Studies of the effect of *Rhizobium* strains on the yield of chick pea. *Bhartiya Krishi Anusandham Patrika*, 8: 147-150.

Tinker, P.B. 1978. Effect of vesicular arbuscular mycorrhiza on plant nutrition and growth. *Physiol. Veg.*, 16: 743-751.