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Effect of Heat-Sterilization and EM (Effective Microorganisms) Application on Wheat (*Triticum aestivum* L.) Grown in Organic-Amended Sandy Loam Soil

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Effective microorganisms (EM), a culture of coexisting beneficial microorganisms predominantly consisting of species of photosynthetic and lactic acid bacteria, yeast and actinomycetes, has been developed by Japanese Scientists. In the present study effect of soil sterilization on performance of a commercial EM biofertilizer (EM Bioab), in improving crop growth and yield in wheat (*Triticum aestivum* L.) was studied in two types of organic amendments viz. farmyard manure (FYM) and *Trifolium alexandrinum* L. green manure (GM). Plant vegetative and reproductive growth and grain yield was better in heat-sterilized than in nonsterilized soil. EM application resulted an insignificant increase in root and shoot dry biomass and grain yield in heat sterilized FYM amended soil while in heat sterilized GM amendment it caused a significant reduction in grain yield. In nonsterilized soils EM application suppressed root and shoot growth at vegetative stage and had insignificant effect at maturity. Effect was more pronounced in FYM than in GM amendment. In nonsterilized FYM amended soil grain yield was also declined by 41% due to EM application.

Keywords: wheat, *Triticum aestivum,* EM (effective microorganisms), soil sterilization, farmyard manure, green manure

Introduction

Upon discovery of chemical fertilizers and pesticides mankind thought of having won the battle against food shortage thereby ensuring survival of the human beings. Role of chemical fertilizers as source of plant nutrients and pesticides in plant protection is beyond doubt. However, indiscriminate use of these chemi-

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cals has often resulted in adverse environmental effects, disturbing the ecological balance of soils and making plants even more susceptible to pests and diseases. The residues of pesticides interrupt natural processes and ecosystems and pose menace to human and animal health. Such critical circumstances had drawn worldwide attention to the need of exploring more intensively the possibilities of increasing agricultural production with less dependency on costly off-farm input in an effort to make our agriculture sustainable. These fundamental concerns have led many agricultural scientists to find alternatives for increasing crop production, to meet food demand and to attain the self sufficiency (Shaxson 2006).

In the recent past some successful efforts have been made to at least partially substitute chemicals with natural substances to minimize the bad effects of the synthetic agrochemicals. One such effort was made by Higa (1991) who isolated some beneficial microorganisms from the soil and called them Effective Microorganisms (EM). EM culture consists of co-existing beneficial microorganisms, the main being the species of photosynthetic bacteria (*Rhodopseudomonas plastris* and *Rhodobacter sphacrodes*); lactobacilli (*Lactobacillus plantarum, L. casei* and *Streptococcus lactis*); yeasts (*Saccharomyces* spp.), and Actinomycetes (*Streptomyces* spp.); which 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). In Pakistan this technology of nature farming was introduced in 1990 by the Nature Farming Research Centre, University of Agriculture, Faisalabad, Pakistan. 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).

There are controversial reports regarding the effect of EM application on crop growth and yield. Many workers have reported increase in crop growth and yield by the application of EM (Daly and Stewart 1999; Yan and Xu 2002; Javaid 2006; Khaliq et al. 2006). However, the investigations of other workers have revealed that the effect of EM on crop growth and yield was usually not evident or even negative especially in the first test crop (Bajwa et al. 1999; Daiss et al. 2008).

Certain soil properties and the indigenous soil microbial populations are often constraints to the establishment of EM cultures. Studies have shown that these constrains can be overcome through periodic repeated applications of EM at least during the first few years (Sangakkara et al. 1998; Javaid et al. 2002). The present study was designed to evaluate the response of indigenous soil microorganisms to EM application in different soil amendment systems. For the purpose EM was applied to wheat grown in farmyard manure and green manure amended heat sterilized and nonsterilized soils.

Materials and Methods

Soil characteristics

Soil used in the experiment was sandy loam in texture having organic matter 0.9%, pH 8.1, EC 4.8 mS cm^{-1}, nitrogen 0.05%, available phosphorus 14 mg kg^{-1} and available potassium 210 mg kg^{-1}. The micronutrients Fe, Cu and Zn were 9.53, 1.71 and 4.42 mg kg^{-1} of soil, respectively.

Soil amendments

Experiment was conducted in earthen pots of diameter 20 cm, each containing 2 kg of soil. Two types of organic amendments viz. farmyard manure (FYM) and *Trifolium alexandrinum* green manure (GM) were mixed in the pot soil @ 5 g 100 g^{-1} and 4 g 100 g^{-1} of soil on dry weight basis. Pots were left for 30 days for decomposition of the manures. After 30 days, pot soil in 50% pots in each of the two amendment systems was heat sterilized at 120 ºC for 12 hours.

EM application

EM stock solution in the commercial name of EM Bioaab was obtained from Nature Farming Research and Development Foundation, Faisalabad, Pakistan. The EM contained high populations of lactic acid bacteria at 1×10^{11} cfu ml^{-1}, photosynthetic bacteria at 1×10^{6} cfu ml^{-1} and yeast 1×10^{3} cfu ml^{-1} of suspension (Higa 2000). The EM stock solution was diluted by adding water in the ratio of 1:1000. Pots containing sterilized and nonsterilized soils were irrigated with 300 ml of dilute solution of EM seven days prior to sowing. The non-EM treatments both in sterilized and nonsterilized soils were used as control.

Treatments

There were four treatments in each of the two soil amendment systems. These include i) – heat sterilized soil (HSS), ii) – HSS+EM, iii) – nonsterilized soil (NSS), and iv) – NSS+EM. Wheat seeds were surface sterilized with 1.0% sodium hypochlorite solution. Five seeds were sown in each pot, which were thinned to 2 uniform seedlings on emergence. Each treatment was replicated three times. Pots were arranged in a completely randomized design on a bench under a wire netting house under natural conditions of light and temperature. Plants were irrigated with tape water whenever required. The EM treated pots also received 300 ml of dilute EM solution in water (1:1000) at fortnight intervals along with irrigation water throughout the experimental period.

Data collection and statistical analysis

Plants were harvested 60 and 150 days after sowing corresponding to vegetative and maturity stages, respectively. The data regarding the number of tillers per plant, root and shoot biomass, and grain yield were recorded. All the data were analyzed statistically by applying ANOVA followed by Duncan's Multiple Range Test (Steel and Torrie 1980) to separate the treatment means.

Results and Discussion

Effect of organic amendments on soil characteristics

After one month of soil amendment with FYM and GM, soil was analyzed for various characteristics. FYM and GM amended soils were characterized with EC 1.9 and 1.4 mS cm^{-1}, pH 8.4 and 8.2, organic matter 1.17 and 1.13%, nitrogen 0.059 and 0.057%, available phosphorus 81.7 and 86.6 mg kg^{-1}, available potassium 644 and 507 mg kg^{-1}, Fe 12.93 and 9.96 mg kg^{-1}, Cu 1.81 and 2.02 mg kg^{-1} and Zn mg kg^{-1}, respectively.

Effect of organic amendments, sterilization and EM on plant vegetative growth

Soil amendments showed a significant effect on root and shoot biomass both at vegetative and maturity stages (Table 1). In general shoot and root biomass was better in FYM than in GM amended soil at vegetative growth stage. At maturity, however, GM amended treatments generally showed greater shoot biomass than that of FYM amended treatments. It could be attributed to different mineralization

Table 1. Analysis of variance for various growth and yield parameters in wheat grown in farmyard and green manure amended heat sterilized and nonsterilized soils, with and without EM application

Trait	Mean squares						
	Soil amendment (A)	Sterilization (S) (E)	EM application	A×S	A×E	S×E	A×S×E
df →	1	1	1	1	1	1	1
60 days after sowing							
Shoot length	7.5 ns	575***	86*	53 ns	5.5 ns	25 ns	1.8 ns
No. of tillers	0.3 ns	20***	6.3**	0.57 ns	1.6 ns	1.9 ns	0.92 ns
Shoot biomass	6***	15.3***	1.1 ns	4.6***	0.01 ns	1.35*	0.48 ns
Root biomass	1.6**	2.1**	0.002 ns	1.4**	0.002 ns	0.42 ns	0.36 ns
150 days after sowing							
Shoot length	0.01 ns	4.5***	0.01 ns	0.09 ns	0.26 ns	0.01 ns	0.26 ns
No. of tillers	0.28 ns	2.9***	0.08 ns	0.9*	0.10 ns	0.08 ns	0.007 ns
Shoot biomass	2.9*	14***	0.25 ns	0.25 ns	0.008 ns	0.02 ns	1.2 ns
Root biomass	1.75*	2.75**	0.01 ns	1.0 ns	0.02 ns	0.46 ns	0.32 ns
No. of spikes	0.01 ns	4.6***	0.01 ns	0.09 ns	0.26 ns	0.01 ns	0.26 ns
Spike biomass	0.12 ns	17***	1.9 ns	0.35 ns	0.59 ns	0.003 ns	3.5*
Spike length	6.7**	1.4 ns	0.15 ns	0.92 ns	2.2 ns	0.92 ns	0.02 ns
Grain yield	0.02 ns	5.8**	1.44 ns	0.75 ns	0.57 ns	0.008 ns	2.2*
No. of grain/spike	147*	0.02 ns	47 ns	164**	95*	4.4 ns	42 ns
100-grain wt.	0.20 ns	0.002 ns	0.12 ns	0.002 ns	0.015 ns	0.08 ns	0.06 ns

*, **, ***, significant at $P \leq 0.05$, 0.01 and 0.001, respectively. ns: non-significant

rates and nutrient availability in the two soil amendment systems at different growth stages of the plant (Kautz et al. 2006).

Soil sterilization also exhibited a significant effect on all the studied parameters of root and shoot growth (Table 1). Root and shoot growth in terms of length and dry biomass, in both the soil amendment systems, was higher in heat sterilized than in nonsterilized soil at both the harvest stages (Table 2). Sterilization removed deleterious microbes and also increased nutrient availability (Linderman 1992). As a result crop growth in heat sterilized soil was increased. Serrasolsas and Khanna (1995) reported an increase in extractable N (organic and mineral forms) after heating to 120 °C. Recently Endlweber and Scheu (2006) have reported an increase in plant growth of *Plantago lanceolata* after heating the soil to100 and 120 °C, due to mobilization of ammonium, nitrate and phosphorus.

EM application showed variable effects in FYM and GM amended heat sterilized soils. In FYM amendment, an insignificant increase of 8% and 11% in shoot dry biomass was recorded at vegetative and maturity stages, respectively, due to EM application. The other plant growth parameters viz. shoot length, number of tillers and root biomass did not exhibited any pronounced response to EM appli-

Table 2. Effect of soil sterilization by heat, farmyard and green manure amendments (50 g kg^{-1} and 40 g kg^{-1} dry soil, respectively), and effective microorganism (EM) application (lactic acid bacteria at 1.5×10^{10} cfu kg^{-1} , photosynthetic bacteria at 1.5×10^{5} cfu kg^{-1} and yeast 1.5×10^{2} cfu kg^{-1} of soil) on the root and shoot growth of wheat (*Triticum aestivum* L.) grown in sandy loam soil

Treatments	Farmyard manure				Green manure			
	Shoot length (cm)	No. of tillers/ Plant	Shoot dry wt. (g)	Root dry wt. (g)	Shoot length (cm)	No. of tillers/ plant	Shoot dry wt. (g)	Root dry wt. (g)
60 days after sowing								
Sterilized soil	52 a	2 a	3.6 a	1.4 ab	47 a	2 a	2.0 a	0.64 a
Sterilized soil + EM	50 a	2 a	3.9 a	1.9 a	46 a	2 a	1.8 a	0.63 a
Nonsterilized soil	42 b	1.5 ab	1.9 b	0.8 ab	42 ab	1 b	1.5 ab	0.55 a
Nonsterilized soil + EM	34 c	1 b	0.7 c	0.3 b	38 b	1 b	0.9 b	0.51 a
150 days after sowing								
Sterilized soil	59 ab	5 a	2.5 ab	0.7 a	62 a	4 a	3.9 a	1.3 a
Sterilized soil + EM	60 a	4 a	2.8 a	0.7 a	57 ab	4 a	3.1 ab	0.5 b
Nonsterilized soil	52 ab	4 a	1.6 ab	0.5 a	59 ab	3 ab	1.7 c	0.4 b
Nonsterilized soil + EM	49 b	3 a	1.1 b	0.4 a	55 b	2 b	1.9 bc	0.5 b

In vertical columns values with different letters show significant difference ($P \leq 0.05$) as determined by DMR Test

cation in heat sterilized FYM amended soil. In contrast to that, in heat sterilized GM amended soil, EM application resulted in 10% and 21% decline in shoot biomass at vegetative and maturity stages, respectively. A similar reduction in root biomass (61%) was also recorded at maturity stage (Table 2). Earlier Bajwa et al. (2002) recorded a positive plant growth response of maize to EM application in FYM but not in GM amended HSS. In a similar study, Javaid et al. (1997) found a significant increase in crop growth and yield in pea in response to EM application in HSS.

In the NSS, EM application adversely affected the root and shoot growth parameters up to 60 days growth in both the organic amendment systems. Effect was significant on shoot length and biomass in FYM amendment. At maturity EM application showed insignificant effect on root and shoot growth in either of the two amendment systems. These results are in line with the findings of some earlier workers who reported that EM application has little or even negative effect on crop growth during the initial period or first year of EM application (Bajwa et al. 1999; Priyadi et al. 2005; Daiss et al. 2008). Generally, crop growth and yield tend to increase gradually by EM application as the subsequent crops are grown (Sangakkara et al. 1998; Javaid et al. 2002). Imai and Higa (1994) stated that the observed decline in crop yields could often be attributed to the fact that soils, where conventional farming is practiced, have become disease-inducing or putre-

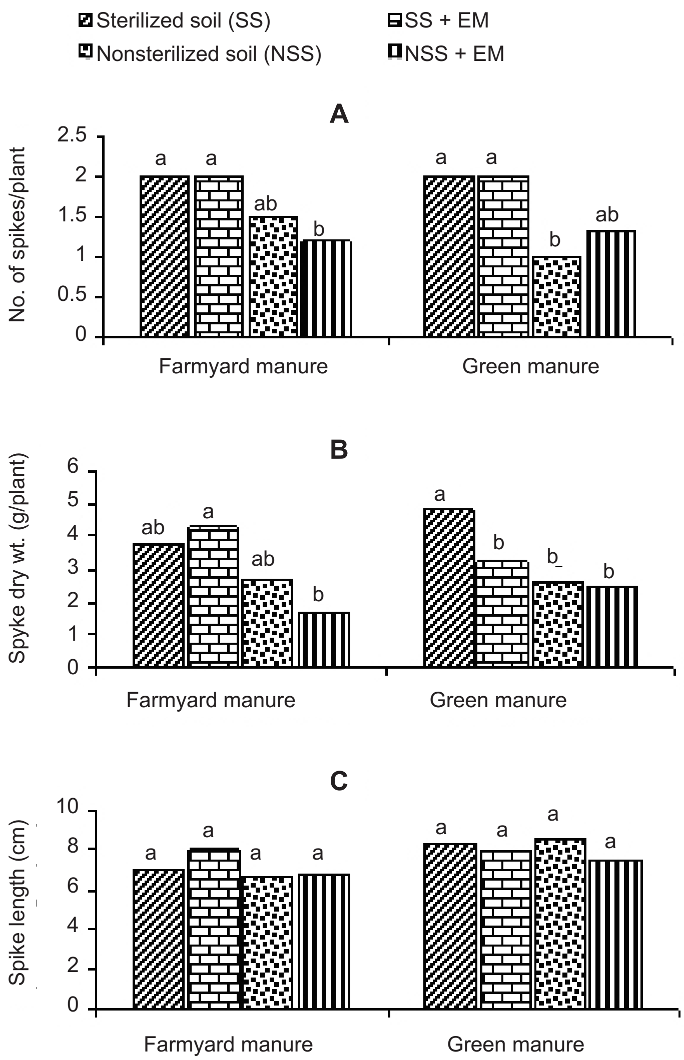

Figure 1 (A–C). Effect of EM (effective microorganisms) application (lactic acid bacteria at 1.5 × 10^{10} cfu kg^{-1}, photosynthetic bacteria at 1.5 × 10^5 cfu kg^{-1} and yeast 1.5 × 10^2 cfu kg^{-1} of soil) and soil sterilization by heat on the different parameters of spike growth in wheat (*Triticum aestivum* cv. Inqalab 91) in a pot experiment with sandy loam, farmyard manure and *Trifolium alexandrinum* green manure amended soils. Values with different letters show significant difference at 5% level of significance as determined by Duncan's Multiple Range Test

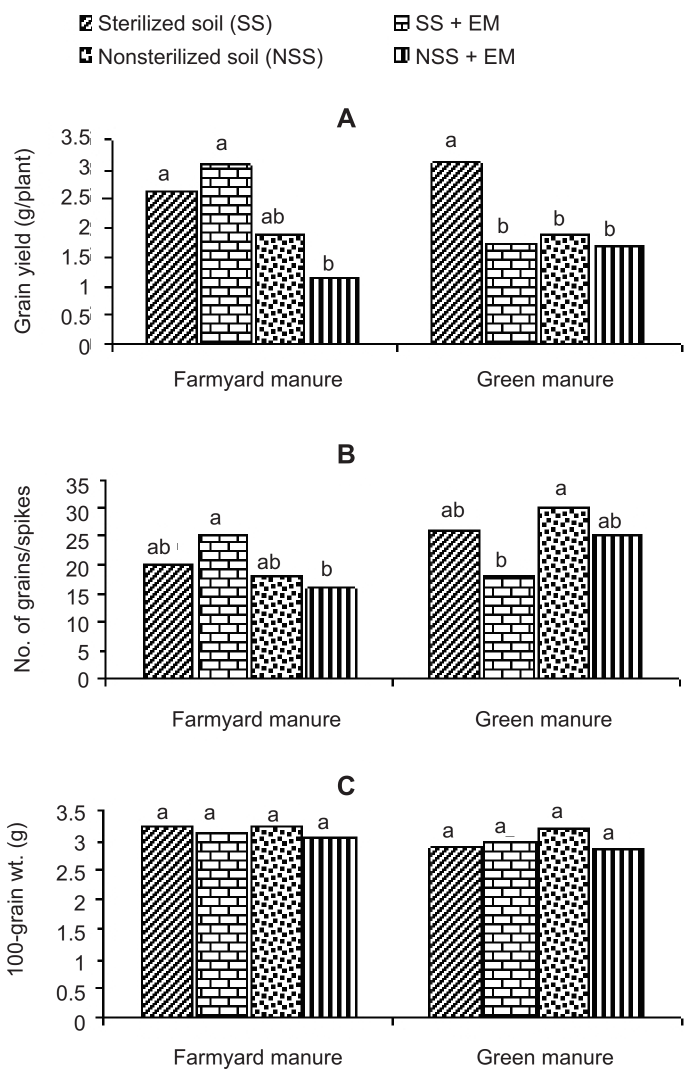

Figure 2 (A–C). Effect of EM (effective microorganisms) application (lactic acid bacteria at 1.5 × 10^{10} cfu kg^{-1}, photosynthetic bacteria at 1.5 × 10^5 cfu kg^{-1} and yeast 1.5 × 10^2 cfu kg^{-1} soil) and soil heat-sterilization on various yield parameters of the wheat (*Triticum aestivum* L. cv. Inqalab 91) in a pot experiment with sandy loam, farmyard manure and *Trifolium alexandrinum* green manure amended soils. Values with different letters show significant difference at 5% level of significance as determined by Duncan's Multiple Range Test

factive 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. However, in contrast to our findings some earlier workers have found positive effects of EM application on crop growth and yield during the first year of EM application (Daly and Stewart 1999; Yan and Xu 2002; Xu et al. 2000). According to Kinjo et al. (2000) the lack of consistency in results of the experiments regarding EM application may be due to variable cultural conditions employed in various studies.

Effect of organic amendments, sterilization and EM on yield

Similar to that of vegetative growth, reproductive growth in terms of number and biomass of spikes, and grain yield was higher in HSS than in NSS (Figs 1 and 2). Response of spike length, number of grains per spike and 100 grain weight to EM application in heat sterilized soil was statistically insignificant. EM application had an insignificant effect on number of spikes both in HSS and NSS in either of the two soil amendment systems (Fig. 1A). EM application failed to induce any pronounced effect on spike and grain biomass in HSS in FYM and in NSS in GM amendment. EM application reduced spike and grain biomass in NSS in FYM and in HSS in GM amendment. Adverse impact of EM application on yield was significant in GM amendment (Figs 1 and 2). Effect of EM application in HSS and NSS on reproductive growth parameters was not parallel to its effect on vegetative growth parameters. The variable response of wheat to EM application in FYM and GM amended soils could be due to the fact that both the microbial biomass and dehydrogenase activity are influenced by the type of organic materials. Generally these parameters are higher in green manure than in farmyard manure amended soils. From the present as well as previous studies no definite conclusion can be drawn regarding the effect of EM application on crop growth and yield. Generally crop response varies with the change of soil type, cropping history, test plant species as well as source and amount of soil nutrients. It, therefore, requires further research to explore the more hidden facts regarding the effect of EM application on growth and yield of crops of economic importance.

References

Bajwa, R., Javaid, A., Rabbani, N. 1999. EM and VAM technology in Pakistan. Effect of organic amendments and EM on VA mycorrhiza, nodulation and crop growth in *Trifolium alexandrianum* L. Pakistan Journal of Biological Sciences **2**:590–593.

Bajwa, R., Javaid, A., Javaid, A. 2002. Effect of soil sterilization, organic amendments and EM application on growth, yield and VA mycorrhizal colonization in maize. Pakistan Journal of Phytopathology **14**:62–67.

Daly, M.J., Stewart, D.P.C. 1999. Influence of "effective microorganisms" (EM) on vegetative production and carbon mineralization – a preliminary investigation. Journal of Sustainable Agriculture **14**:15–25.

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

Endlweber, K., Scheu, S. 2006. Establishing arbuscular mycorrhiza-free soil: A comparison of six methods and their effects on nutrient mobilization. Applied Soil Ecology **34**:276–279.

Higa, T. 1991. Effective microorganisms: A biotechnology for mankind. In: Parr, J.F., Hornick, S.B., Whitman, C.E. (eds), Proceedings of 1st Kyusei Nature Farming. USDA, Washington, D.C. USA, October 17–21, 1989, pp. 8–14.

Higa, T. 2000. What is EM technology? EM World Journal **1**:1–6.

Hussain, T, Javaid, T., Parr, J.F., Jilani, G., Haq, M.A. 1999. Rice and wheat production in Pakistan with effective microorganisms. American Journal of Alternative Agriculture **14**:30–36.

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

Imai, S., Higa, T. 1994. Kyusei nature farming in Japan. Effect of EM on growth and yield of spinach. In: Proceedings of 2nd International Conference on Kyusei Nature Farming, Brazil, Oct. 7–11, 1991, pp. 92–96.

Javaid, A. 2006. Foliar application of effective microorganisms on pea as an alternative fertilizer. Agronomy for Sustainable Development **26**:257–262.

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

Javaid, A., Anjum, T., Bajwa, R. 2002. EM and VAM technology in Pakistan. XII. Growth, nodulation and VA mycorrhizal response of *Phaseolus vulgaris* to long-term EM application. Pakistan Journal of Phytopathology **14**:57–61.

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

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

Kinjo, T., Perez, K. de Almeida, E, de Oliveia, J.O. 2000. Plant growth affected by EM Bokashi and chemical fertilizers Nature Farming and Environment **1**:33–38.

Linderman, R.G. 1992. Vesicular arbuscular mycorrhiza and soil microbial interactions. In: Bethlenfalvay, G.J., Linderman, R.G. (eds.), Mycorrhiza in Sustainable Agriculture. ASA Special Publication No. 54. American Society of Agronomy, Inc. USA. 49 pp.

Priyadi, K., Hadi, A., Siagian, T.H., Nisa, C., Azizah, A., Raihani, N., Inubushi, K. 2005. Effect of soil type, application of chicken manure and effective microorganisms on corn yield and microbial properties of acidic wetland soils in Indonesia. Soil Science & Plant Nutrition **51**:689–691.

Sangakkara, U.R., Marambe, B., Attanayake, A.M.U., Piyadasa, E.R. 1998. Nutrient use efficiency of selected crops grown with effective microorganisms in organic systems. In: Proceedings of 4th International Conference on Kysei Nature Farming held in Paris, France, June 19–21, 1995, pp. 111–117.

Serrasolsas, I., Khanna, P.K. 1995. Changes in heated and autoclaved forest soils of S.E. Australia. I. Carbon and nitrogen. Biogeochemistry **29**:3–24.

Shaxson, T.F. 2006 Re-thinking the conservation of carbon, water and soil: a different perspective Agronomy for Sustainable Development **26**:9–19.

Steel, R.G.D., Torrie, J.H., 1980. Principles and procedures of statistics. A Biometrical Approach. 2nd edition. McGraw Hill Book Co. Inc. New York, USA, pp. 107–109.

Xu, H.L., Wang, R., Mridha, M.A.U. 2000. Effect of organic fertilizers and a microbial inoculant on leaf photosynthesis and fruit yield and quality of tomato plants. Journal of Crop Production **3**:173–182.

Yan, P.S., Xu, H.L. 2002. Influence of EM Bokashi on nodulation, physiological characters and yield of peanut in nature farming fields. Journal of Sustainable Agriculture **19**:105–112.