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Field evaluation of effective microorganisms (EM) application for growth, nodulation, and nutrition of mung bean

Arshad JAVAID*, Rukhsana BAJWA

Institute of Plant Pathology, University of the Punjab, Quaid-e-Azam Campus Lahore 54590, PAKISTAN

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Abstract: Effective microorganisms (EM) is a commercial biofertilizer that contains a mixture of co-existing beneficial microorganisms collected from natural environments. Predominantly it consists of species of photosynthetic and lactic acid bacteria, yeast, and actinomycetes. The present study was conducted to evaluate the effect of EM application on growth, nodulation, yield, and nutrient uptake in mung bean [*Vigna radiata* (L.) Wilczek] var. NIAB Mung 98 under field conditions. Field soil was amended with farmyard manure at 20 t ha^{-1}, *Trifolium alexandrinum* green manure at 20 t ha^{-1}, and recommended (NPK) and half (½ NPK) doses of chemical fertilizers. EM was applied in the form of a dilute solution in water (1:1000) at fortnight intervals throughout the experiment. EM application significantly enhanced shoot biomass in farmyard manure, ½ NPK and NPK amendments. Similarly, EM significantly increased grain yield by 24% and 46% in farmyard manure and NPK fertilizers amendments, respectively. By contrast, in green manure amendment, EM application resulted in a significant decline of 23% in grain yield. In ½ NPK amendment, the effect of EM application on grain yield was insignificant. Nodulation in terms of number and biomass of nodules was significantly suppressed by EM application in farmyard manure and green manure amendments. In NPK amendment, a significant increase in nodule biomass was recorded due to EM application. EM significantly enhanced nitrogen, phosphorus and potassium nutrition of the test plant in farmyard manure amendment both at flowering stage and maturity. However, in NPK amended soil, EM application markedly enhanced plant nutrition at later growth stage only.

Key words: Effective microorganisms (EM), NPK fertilizers, organic amendments, *Vigna radiata*

Introduction

EM stands for Effective Microorganisms and was developed at the University of the Ryukyus, Okinawa, Japan, in the early 1980s by Prof. Dr. Terou Higa. The expansion process of EM Technology began in 1989 with the inception of the 1st International Kyusei Nature Farming Conference in Thailand, where the need to scientifically validate this technology and to enhance its use was discussed. Consequently, Asia Pacific Natural Agriculture Network (APNAN) was founded. It included 13 countries ranging from the west coast of the USA through Asia to Pakistan (Hussain et al. 2000). EM is a fermented mixed culture of naturally occurring species of co-existing microorganisms in acidic medium (pH below 3.5). Among the main microorganisms in EM culture are the species of photosynthetic bacteria (*Rhodopseudomonas plastris* and *Rhodobacter*

* E-mail: arshadjpk@yahoo.com

sphacrodes), lactobacilli (*Lactobacillus plantarum*, *L. casei*, and *Streptococcus lactis*), yeasts (*Saccharomyces* spp.), and actinomycetes (*Strptomyces* spp.). Microorganisms in EM improve crop health and yield by increasing photosynthesis, producing bioactive substances such as hormones and enzymes, accelerating decomposition of organic materials and controlling soil-borne diseases (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. 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).

Previous studies on EM application have revealed that plant growth in EM applied treatments was just as good or better, and quality of plant products was superior to conventional farming (Daly and Stewart 1999; Iwaishi 2000; Xu 2000; Yamada and Xu 2000; Javaid 2006, 2009; Khaliq et al. 2006). However, experiences of some researchers revealed that the effect of EM on crop yield was usually not evident or even negative particularly in the first test crop (Daiss et al. 2008; Javaid et al. 2008, Javaid and Shah 2010). It is often difficult to establish the predominance of effective microorganism cultures in soil with only a single application and during only one season. Indigenous soil microbial populations are often constraints to the establishment of these microorganisms (Bajwa et al. 1995). However, these constrains can be overcome through periodic repeated applications of effective microorganisms at least during the first few years (Javaid et al. 2000). Efficacy of effective microorganisms is also affected by soil types. Furthermore, source and amount of soil nutrients as well as test crop may affect the establishment and efficacy of these microorganisms when application of these microorganisms is started in a soil for the first time (Bajwa et al. 1999, Javaid et al. 2002; Javaid 2010). The present research work was, therefore, carried out to study the effect of EM application on crop growth, yield, nodulation, and nutrition in mung bean [*Vigna radiata* (L.) Wilczek] in different soil amendment systems, under field conditions.

Materials and methods

Soil characteristics

A field experiment was conducted at Botanical Garden, University of the Punjab Lahore, Pakistan. The field soil was sandy loam in texture having organic matter 0.9%, pH 8.2, total nitrogen 0.05%, available phosphorus 14 mg kg^{-1}, and available potassium 210 mg kg^{-1}. The micronutrient B, Mn, Fe, Cu, and Zn were 1.06, 22.8, 10.8, 1.9, and 1.3 mg kg^{-1} of soil, respectively (Richards 1954).

Soil amendments

Fresh farmyard manure (FYM) at 20 t ha^{-1} was thoroughly mixed in the field plots. The farmyard manure was moistened with dilute EM solution (1:1000) before mixing in the respective EM treated plots. The plots were irrigated with tap water. The plots that received EM treated farmyard manure were also supplied with EM dilute solution at 2 L m^{-2}. Plots were left for 40 days to decompose the manure and irrigated whenever required to maintain the soil moisture.

T. alexandrinum green manure (GM), grown in the respective plots, was thoroughly mixed in the soil at 20 t ha^{-1}. Before mixing in the soil, green manure was sprayed with dilute EM solution in the respective plots. Plots were irrigated with tap water and EM dilute solution was applied as in FYM amended soil, and left for 40 days for decomposition of the manure.

Two levels of mineral NPK fertilizers were applied in the experiment. In recommended NPK fertilizer treatment, a basal dose of N at 10 kg ha^{-1} as urea, P_2O_5 at 25 kg ha^{-1} as triple supper phosphate and K_2O at 25 kg ha^{-1} as K_2SO_4 were mixed in the soil 3 days before sowing and irrigated with tap water. N at 7 kg ha^{-1} as urea was also top dressed 40 days after sowing at 50% flowering stage, after taking the first harvest. In a similar experiment, half the doses of NPK fertilizers were applied. EM application in the respective plots was started 40 days prior to the sowing of seeds as in case of GM and FYM amended soils.

Experimental design

Experiment was conducted in a split plot design using 3 replications. Main plot treatment was EM application (present or absent) while soil amendments were kept as subplots of 1.5 × 2.0 m.

Seeds of *V. radiata* var. NM-98 were surface sterilized with 1% sodium hypochlorite for 3 min followed by several washings with sterilized water. Seeds were sown in longitudinal rows with intra- and inter-row spacing of 18 and 30 cm, respectively. There were 6 rows in each subplot with 6 plants in each row. Each treatment was replicated 3 times.

EM application schedule

EM stock solution was obtained from Nature Farming Research Centre, University of Agriculture, Faisalabad, Pakistan. EM solution consisted of mixed culture of beneficial microorganism including a predominant population of lactic acid bacteria (1×10^8 cfu mL^{-1}) and yeast (2×10^6 cfu mL^{-1}, and a small proportion of photosynthetic bacteria (1×10^3 cfu mL^{-1}) (Sajjad et al. 2003). EM is available in a dormant state and requires activation before application. Activation involved the preparation of a solution containing 1 part EM stock solution + 1 part of 1% sugar solution + 20 parts water by volume. The activation process took place away from direct sunlight for 3 days. The activated EM solution was diluted to 1:1000 (compared to original EM solution) by adding sterilized water. The respective EM treated plots received dilute EM solution (1:1000) at 2 L m^{-2} throughout the experimental period at fortnight intervals.

Data collection and plant analysis

Plants were harvested 40 and 75 days after sowing at flowering and maturity stages, respectively. At flowering stage, alternative plants were harvested from each row so that more space would be available to the remaining growing plants. At each harvest, plants were uprooted along with the rhizospheric soil. Data regarding dry biomass, number and biomass of nodules, and yield were recorded.

Shoot nitrogen was estimated by Kjeldahl's method. To 0.1 g of thoroughly crushed plant materials, 3 mL of H_2SO_4 and 1.0 g of digestion mixture (K_2SO_4: $CuSO_4$: selenium metal = 1:1:0.5) were added. Materials were digested on electric heater in a Kjeldahl digestion flask for 3 h and cooled. The digested samples were cooled and the volume was made up to 100 mL. From this, a 10 mL aliquot was taken for distillation. Fifteen milliliters of 50% NaOH was poured into the distillation flask. A conical flask containing 10 mL of boric acid cum indicator solution was placed under the condenser. The distillation was continued until the distillation amounted to 35 mL. The contents of the flask were titrated against N/100 H_2SO_4 to get back the original color of the boric acid indicator solution (Jackson 1962). The N content of the samples was determined by applying the following formula:

1 mL of N/100 H_2SO_4 = 0.14 mg N

For estimation of shoot P and K content, 1.00 g of dried plant material was mixed in 20 mL of conc. HNO_3. The flask was heated until solid particles nearly disappeared. The contents in the flask were cooled and 10 mL of 72% perchloric acid ($HClO_4$) was added. The resulting mixture was heated gently at first and then more vigorously until a clear colorless solution resulted. Digested material was transferred to a 100 mL volumetric flask after cooling and the volume made up to the mark. The flask was thoroughly shaken, allowed to stand overnight, and filtered. A 5 mL aliquot was taken in a 50 mL volumetric flask and 5 mL of each of ammonium vanadate (0.25%), H_2SO_4, and ammonium molybdate (5%) were added and left for 30 min (Jackson 1962). P and K contents were estimated by spectrophotometer and flam photometer, respectively.

All the data were tested by analysis of variance (ANOVA) and means were compared by Duncan's multiple range test at 5% level of significance (Steel and Torrie 1980) using SPSS and COSTAT.

Results

Effect of soil amendments and EM on shoot growth

Analysis of variance revealed that the effect of growth stage (G) and soil amendment (A) was significant while that of EM was insignificant for shoot length. The interactive effect of G × A, A × EM, and G × A × EM was also significant while that of G × EM was insignificant for this studied parameter (Table 1). At the flowering stage, maximum shoot length was recorded in ½ NPK treatment, which was significantly greater than shoot length in farmyard manure (FYM) and green manure (GM) amendments. At maturity, the highest shoot length was obtained in FYM followed by GM, which were significantly

higher than shoot length in the 2 mineral fertilizers treatments (Table 2). EM application significantly enhanced shoot length in FYM at the flowering stage. In GM amendment, EM application significantly reduced shoot length at both the harvest stages. In ½ NPK as well as in recommended NPK treatments, the effect of EM application was insignificant at the flowering stage as well as at maturity (Table 2).

The effect of growth stage, soil amendments, and EM as well as their various interactions was significant for shoot dry biomass (Table 1). At the flowering stage, the highest shoot biomass was recorded in FYM followed by in NPK amendment. However, at maturity maximum shoot biomass was recorded in GM followed by in FYM, NPK, and ½ NPK amendments, in that order (Table 2). EM application significantly enhanced shoot biomass in FYM and ½ NPK amendments at both the flowering and maturity stages. Similarly, EM application significantly enhanced shoot biomass in NPK amendment at maturity after an insignificant effect at the flowering stage. In contrast, in GM amendment, a significant suppression in shoot biomass was evident at both the flowering and maturity stages (Table 2).

Effect of soil amendments and EM on root growth

The effect of growth stage and soil amendments was significant while that of EM was insignificant for root biomass as shown by analysis of variance. Interactive effects of G × A, A × EM, and G × A × EM were also significant for root biomass but that of G × EM was not (Table 1). At the flowering stage, the effect of EM application was insignificant in all except GM amendment, where a significant reduction in the studied parameter was recorded. However, at maturity, EM application significantly enhanced root biomass in both the mineral fertilizer treatments. Conversely, in both the organic amendments (GM and FYM), the effect of EM was insignificant (Table 2).

Effect of soil amendments and EM on nodulation

The effect of growth stage was significant for number as well for fresh and dry biomass of nodules. The effect of soil amendments was only significant for nodule number and that of EM was significant for number and fresh biomass of nodules (Table 1). At the flowering stage, maximum number of nodules (22 per plant) was recorded in FYM followed by 19 in GM, 18 in NPK, and 12 in ½ NPK amendment. EM application significantly reduced number and biomass of nodules in GM and FYM amended soils. The effect of EM application on nodulation was insignificant in ½ NPK. However, in NPK amendment, a significant increase in dry biomass of nodules was evident. Nodulation at maturity was very poor in all the treatments because of decomposition of nodules (Table 2).

Effect of soil amendments and EM on yield

The effect of A, EM, and A × EM was significant for number of pods and grain yield and insignificant for pod length and number of seeds per pod (Table 3). Maximum number of pods per plant, i.e. 125, was recorded in GM followed by 107 in FYM, 81 in ½ NPK, and 78 in NPK amendment. The difference in number of pods between organic and mineral fertilizers amendments was significant. EM application significantly enhanced pod number in FYM and NPK amendment. In ½ NPK amendment, the effect of EM application was insignificant while in GM amendment a significant reduction in pod number was recorded due to EM application (Figure 1a).

Grain yield in different soil amendments was in the order of FYM > GM > ½ NPK > NPK. The effect of EM application in different soil amendments was similar to that of the effect on number of pods. EM application significantly enhanced grain yield in FYM and NPK amendments by 39% and 57%, respectively. Conversely, in GM amendment, there was a significant decrease of 23% in yield due to EM application (Figure 1b).

Effect of soil amendments and EM on nitrogen nutrition

Analysis of variance showed that the effect of growth stage, soil amendments, EM application, and their interactions was significant for shoot N concentration and total N content (Table 1). At the flowering stage, the highest shoot N concentration was recorded in GM amended soil, which was significantly greater than N concentration in FYM and ½ NPK amendments. However, at maturity, N concentration was in the order of ½ NPK > NPK >

Table 1. Analysis of variance for various growth, nodulation and nutrition parameters of mung bean as affected by soil amendments and effective microorganism application at 2 growth stages.

Trait	df	Mean squares											
		Shoot length	Shoot dry biomass	Root dry biomass	Nodule no.	Nodule fresh biomass	Nodule dry biomass	N conc.	N content	P conc.	P content	K conc.	K content
Treatments	15	264***	717***	13***	186***	10146***	441***	3.4***	526540***	9.8***	840***	93***	38130***
Growth stage (G)	1	1842***	7964***	162***	2275***	98555***	4219***	42***	4230468***	125***	186*	1215***	113102***
Amendment (A)	3	195***	262***	2.08**	38**	1073^{ns}	46^{ns}	0.53***	149570***	2.87***	2739***	24.61***	79723***
EM	1	0.03^{ns}	246***	0.59^{ns}	87***	5146**	96^{ns}	0.17^{ns}	418880***	0.403***	297**	11.73***	41654***
G × A	3	191***	183***	1.87**	13^{ns}	1150^{ns}	138**	1.35***	182109***	2.88***	118*	11.82***	24294***
G × EM	1	0.007^{ns}	129**	1.6^{ns}	92***	5525**	75^{ns}	0.021^{ns}	135256***	0.003^{ns}	23^{ns}	2.18^{ns}	6533*
A × EM	3	234***	251***	3.3***	31**	6301***	307***	0.526***	476302***	0.438***	756***	12.60***	31954***
G × A × EM	3	86*	109**	2.4**	30**	5797***	249***	0.790***	229851***	1.017***	418***	5.92***	916^{ns}
Error	32	29	20	0.47	7	587	26	0.078	6170	0.027	42	0.586	1175
Total	48												

*, **: ***, Significant at P ≤ 0.05, P ≤ 0.01, and 0.001, respectively. ns: Non-significant.

Table 2. Effect of soil amendments and EM application on plant growth and nodulation of mung bean under field conditions.

Soil amendments	EM	Flowering stage						Maturity stage					
		Shoot length (cm)	Shoot biomass (g)	Root biomass (g)	Nodule number	Nodules fresh biomass (mg)	Nodules dry biomass (mg)	Shoot length (cm)	Shoot biomass (g)	Root biomass (g)	Nodule number	Nodules fresh biomass (mg)	Nodules dry biomass (mg)
Farmyard manure	–	41 d	7.9 b	0.72 a	22 a	135 ab	17 b	63 a	35 bc	5.7 a	2 b	17 b	4 ab
	+	55 a	13.5 a	0.66 a	12 b-d	54 c	9 b	63 a	52.5 a	5.3 a	5 a	28 a	6 a
Green manure	–	41 d	5.5 c	0.65 a	19 ab	188 a	35 a	62 a	43 ab	5.3 ab	2 b	10 c	3 ab
	+	28 e	2.4 d	0.22 b	8 d	44 c	8 b	50 b	29 c-e	3.3 bc	0.3 bc	1 d	0.3 b
½ NPK	–	50 ab	4.2 bc	0.77 a	12 cd	77 bc	18 b	51 b	19.5 e	2.3 c	0 c	0 d	0 b
	+	48 a-c	8.0 b	0.65 a	13 b-d	80 bc	18 b	54 ab	26 b-d	5.1 ab	0.1 bc	1 d	0.3 b
NPK	–	47 b-d	7.3 bc	0.62 a	18 a-c	75 bc	17 b	50 b	20.3 de	2.7 c	0 c	0 d	0 b
	+	47 b-d	8.9 b	0.67 a	13 bc	132 ab	36 a	59 ab	43 bc	4.7 ab	0 c	0 d	0 b

– EM not applied + EM applied In a column values with different letters show a significant difference (P ≤ 0.05) as determined by Duncan's multiple range test.

Table 3. Analysis of variance for various yield parameters of mung bean as affected by soil amendments and EM application.

Trait	df	Mean squares			
		No. of pods	Pod length	No. of seeds pod^{-1}	Yield
Treatments	7	4156***	0.20^{ns}	0.93^{ns}	273***
Amendment (A)	3	4899***	0.242^{ns}	0.70^{ns}	404***
EM	1	1717**	0.003^{ns}	1.35^{ns}	137**
A × EM	3	4227***	0.22^{ns}	1.02^{ns}	189***
Error	16	126	0.23	0.47	18
Total	24				

,*, Significant at P ≤ 0.01 and 0.001, respectively. ns: Non-significant.

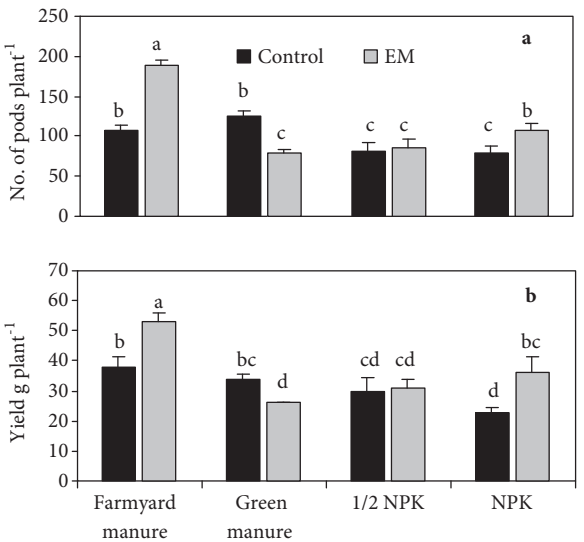

Figure 1. Effect of soil amendments and EM application on number of pods (A) and grain yield (B) of mung bean under field conditions. Vertical bars show standard error of means of 3 replicates. Values with different letters show significant difference (P ≤ 0.05) as determined by Duncan's multiple range test.

GM > FYM (Table 4). EM application significantly enhanced N concentration in FYM and ½NPK amendments at the flowering stage, and in NPK amendment at maturity. Conversely, at maturity EM application significantly suppressed this studied parameter in GM and ½ NPK amendments (Table 4).

Maximum total shoot N content at the flowering stage was recorded in FYM, followed by in NPK, ½ NPK, and GM amendment, in that order. At maturity, N content was highest in GM amendment, which was significantly higher than N content in all other amendments (Table 4). EM application significantly increased N content in FYM and NPK amendment at both the flowering and maturity stages. A similar increase was also recorded due to EM application in ½ NPK at the flowering stage. In contrast, in GM amendment, EM application significantly suppressed N content at the flowering as well as the maturity stage (Table 4).

Effect of soil amendments and EM on phosphorus nutrition

Analysis of variance revealed that the effect of growth stage, soil amendments, and EM application as well as interactions of G × A, A × EM, and G × A × EM was significant for both shoot P concentration and total P content (Table 1). At the flowering stage, the highest shoot P concentration was recorded in GM, followed by FYM, NPK, and ½ NPK amendment, in that order. Differences among the various soil amendments were significant. At maturity, maximum shoot P concentration was recorded in FYM amendment, which was significantly greater than P concentration in all other soil amendments (Table 4). EM application significantly enhanced P

Table 4. Effect of soil amendments and EM application on NPK concentration and content in mung bean under field conditions.

Soil amendments	EM	Flowering stage						Maturity stage					
		N conc. (mg g^{-1})	N content (mg $plant^{-1}$)	P conc. (mg g^{-1})	P content (mg $plant^{-1}$)	K conc. (mg g^{-1})	K content (mg $plant^{-1}$)	N conc. (mg g^{-1})	N content (mg $plant^{-1}$)	P conc. (mg g^{-1})	P content (mg $plant^{-1}$)	K conc. (mg g^{-1})	K content (mg $plant^{-1}$)
Farmyard manure	–	45 bc	355 cd	4.98 c	39 b	14 c	110 bc	20 e	696 c	1.62 a	56 a	8.3 b	286 b
	+	53 a	710 a	5.93 a	80 a	21 a	276 a	24 de	1257 b	1.12 b-d	59 a	9.8 a	512 a
Green manure	–	52 a	287 e	5.57 b	31 bc	17 b	95 c	29 c	1263 b	0.92 cd	40 b	5.3 c	228 bc
	+	46 bc	110 f	4.33 d	10 d	18 b	42 d	28 cd	795 c	0.86 d	26 cd	5.6 c	171 cd
½ NPK	–	44 c	318 de	3.44 f	25 c	17 b	122 bc	34 ab	670 c	1.33 b	26 cd	5.2 c	101 d
	+	50 ab	410 b	3.59 f	29 bc	17 b	136 b	28 cd	681 c	1.19 bc	38 bc	5.6 c	176 cd
NPK	–	47 a-c	340 d	3.99 e	28 bc	14 c	105 bc	31 bc	621 c	1.13 b-d	23 d	5.4 c	110 d
	+	44 bc	393 bc	3.33 f	29 bc	13 c	118 bc	39 a	1660 a	1.16 bc	36 b-d	5.4 c	194 c

– EM not applied + EM applied In a column values with different letters show a significant difference ($P \leq 0.05$) as determined by Duncan's multiple range test.

concentration in FYM amendment at the flowering stage. By contrast, in GM and NPK amendments, EM application significantly suppressed P concentration while in ½ NPK amendment its effect was insignificant. At maturity, the effect of EM application on shoot P concentration was insignificant in all 4 soil amendment systems (Table 4).

At both the growth stages, the highest shoot P content was recorded in FYM, followed by GM amendment. EM application significantly enhanced shoot P content in FYM amendment at both the growth stages. Similarly, in ½ NPK and NPK amendments, a marked increase in P content was also recorded due to EM application at maturity. In contrast, EM application in GM amendment resulted in a consistent and significant reduction in this studied parameter (Table 4).

Effect of soil amendments and EM on potassium nutrition

The effect of growth stage, soil amendments, and EM application was significant for both shoot K concentration and total K content. All the interactive effects of these variables were also significant for these studied parameters except the effect of G × EM for K concentration and that of G × A × EM for K content (Table 1). At the flowering stage, the highest shoot K concentration was recorded in GM and ½ NPK amendments, which were significantly greater than shoot K concentration recorded in FYM and NPK amendments. However, at maturity maximum K concentration was recorded in FYM, which was significantly greater than that recorded in other soil amendments (Table 4). EM application significantly enhanced P concentration in FYM amendment at both growth stages. In other soil amendment systems, the effect of EM application was insignificant (Table 4).

Difference in shoot K content among the soil amendments was insignificant at the flowering stage. However, at maturity, K content in both the organic matter amended soils was significantly higher as compared to K content in both the mineral fertilizers amendments. EM application significantly enhanced shoot K content in FYM amendment at both growth stages. A similar effect of EM application was also recorded in ½ NPK and NPK amendments at maturity. In contrast, EM application in GM amendment adversely affected the K content. The effect was more pronounced and significant at the flowering than at the maturity stage (Table 4).

Discussion

EM application exhibited variable effects on plant vegetative and reproductive growth in different soil amendment systems. A significant increase in shoot biomass, number of pods and grain yield was recorded due to EM application in farmyard manure as well as in soil amended with the recommended dose of NPK fertilizers. These results support the findings of Khaliq et al. (2006), who reported that EM application either in combination with organic matter or mineral NPK significantly enhanced cotton yield as compared to the treatments where these soil amendments were used without EM application. Similarly, Hussain et al. (1999) found an increase in wheat and rice grain yield when EM application was carried out in combination with farmyard manure or mineral NPK. The higher grain yield in the present and earlier studies, when EM was applied in combination with organic matters, can be attributed largely to the activity of the introduced beneficial microorganisms, which enhanced the decomposition of organic materials and the release of nutrients for plant uptake (Hussain et al. 1999). However, the fact that EM also increased grain yield when applied with recommended dose of NPK fertilizers suggests that EM may have induced other mechanisms that exert a positive effect on the yield (Higa and Widdana 1991). The enhanced crop growth and yield can possibly be attributed to activity of photosynthetic bacteria such as *Rhodopseudomonas palustris* and *Rhodobacter sphaeroides* present in EM solution. These bacteria are a group of independent, self-supporting microbes. They synthesize useful substances from secretions of plant roots, organic matter and harmful gases such as hydrogen sulfide, by using sunlight and the heat of soil as sources of energy (Kim et al. 2004). The useful substances produced by these bacteria include amino acids, polysaccharides, nucleic acids, bioactive substances, and sugars, all of which promote plant growth and development. The metabolites developed by these microbes are absorbed directly by plants (Higa 2000; Kin and Lee 2000; Ranjith et al. 2007). In the present study, EM application significantly enhanced shoot biomass in ½ NPK amendment

but its effect on reproductive growth was not very pronounced. In green manure amendment, EM application generally suppressed both vegetative and reproductive growth of the test crop. In contrast, earlier researchers reported enhanced crop growth and yield due to EM application in combination with green manure (Hussain et al. 1999). In the present study, the soil surface became hard due to EM application in green manure amended soil, which resulted in reduced crop growth and yield. The reason for this hardness of the soil surface is not known.

Nodulation data at the flowering stage were more reliable than at maturity because at the later growth stage most of the nodules were decomposed. Nodulation was generally better in organic matter amended soils than in mineral fertilizer amendments. EM application at the flowering stage significantly reduced the number and biomass of nodules in farmyard as well as in green manure amended soils. The effect of EM application on nodulation was insignificant in ½ NPK. Conversely, in NPK amendment, EM application significantly increased nodules' dry biomass. Results of previous studies regarding the effect of EM application on nodulation in legumes are highly variable, ranging from significantly negative to significantly positive (Javaid et al. 2000, 2002; Javaid 2006; Khan et al. 2006). This variation in nodulation could be attributed to various factors including the soil physical and chemical properties, soil amendment, cropping and agricultural practices history, soil indigenous rhizobial and other microbe's population, environmental conditions of the area, and concentration of EM.

In the present study, generally the effect of EM application on shoot nutrition was different at the 2 growth stages. It could be attributed to the fact that at the later growth stage some nutrients were transferred to grains, which were not taken into consideration as only shoot N, P, and K concentrations and contents were estimated. In general, EM application enhanced shoot N, P, and K nutrition at one or the other growth stage in the soils amended with farmyard manure. Similar effects of EM application on plant nutrition in organic amended soils have also been reported in wheat, rice, cotton, capsicum (*Capsicum annuum* L.), and cowpea (*Vigna unguiculata* L.) (Sangakkara et al. 1998; Hussain et al. 1999; Khaliq et al. 2006). Effective microorganisms enhance the degradation and chemical breakdown of organic materials and stimulate the process of mineralization of organic matter (Hussain et al. 1999), releasing more nutrients into the soil-plant system (Daly and Stewart 1999). Lactic acid bacteria in EM culture are especially responsible for decomposition of materials such as lignin and cellulose in the organic materials (Gao et al. 2008). In GM amendment, however, the effect of EM application was generally insignificant or negative. It could be attributed to poor plant growth in these plots due to the hard soil surface in EM treated GM amended plots. In both the mineral fertilizers amendments, EM application markedly enhanced shoot N, P, and K content. Similar effects of EM application on uptake of nutrients in mineral fertilizers amended soil have also been reported for rice and cotton crops (Hussain et al. 1999; Khaliq et al. 2006). It may possibly be due to rapid decomposition of organic matter already present in the soil. Furthermore, there may have been other EM-induced mode of actions such as enhanced nutrient uptake efficiency of the plants (Sangakkara et al. 1998).

In conclusion, for better plant growth, yield, and nutrition in mung bean under field conditions, EM application should be carried out in combination with farmyard manure or the recommended dose of mineral NPK fertilizers.

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