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Research Article

Growth, nodulation and yield of black gram [*Vigna mungo* (L.) Hepper] as influenced by biofertilizers and soil amendments

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Abstract

EM (effective microorganisms) is a commercial biofertilizer mainly consists of photosynthetic and lactic acid bacteria, yeast and actinomycetes. The present study was undertaken to investigate the effect of EM application and two strains of nitrogen fixing *Bradyrhizobium japonicum* (TAL- 102 and MN-S) on plant growth, nodulation and yield of black gram [*Vigna mungo* (L.) Hepper] in different soil amendment systems including unamended soil, farmyard manure (FYM) @ 5 g 100 g-1, *Trifolium alexandrinum* green manure (GM) @ 4 g 100 g-1 and recommended dose of NPK fertilizers. Nodule number was significantly enhanced by inoculation of either of the two *B. japonicum* strains in NPK and un-amended soils. A marked increase in nodule biomass was also recorded due to *B. japonicum* inoculation in these 2 types of soils. Grain yield was significantly increased by 46% due to either of the two *B. japonicum* strains in NPK amended soil. EM application markedly enhanced nodule number in FYM amended soil. Conversely, EM application in combination with either of the two *B. japonicum* strains resulted in pronounced reduction both in number and biomass of nodules in NPK fertilizers amendment. EM application significantly enhanced grain yield by 48% in NPK amendment without *B. japonicum* inoculation.

Keywords: Black grams, *Bradyrhizobium japonicum*, effective microorganisms, nitrogen fixation, soil amendments.

Introduction

Chemical fertilizers are an indispensable component of today's agriculture. About 60% of humanity eventually owes its nutritional survival to N fertilizers (Fixon and West, 2002). However, growing concern about the environmental consequences of mineral N use and its future cost perspectives emphasize the need to develop new production technologies that are sustainable both economically and ecologically (Khaliq et al., 2006). Organic materials hold great promise as a source of multiple nutrients and ability to improve soil characteristics (Soumare et al., 2003; Moller, 2009). Since the effect of organic nutrients on crop yield is long term and not immediate, thus farmers are reluctant to use organic fertilizers in their cropping system. Use of EM (effective microorganisms) along with organic materials possibly be an effective technique for stimulating release of nutrients from organic sources. EM technology was developed by Dr. Teuro Higa in 1970's at the University of Ryukyus, Okinawa, Japan. Effective microorganisms culture consists of co-existing beneficial microorganisms, the main being the species of photosynthetic bacteria; *Rhodopseudomonas plastris* and *Rhodobacter sphacrodes*; lactobacilli such as *Lactobacillus plantarum*, *L. casei* and *Streptococcus lactis*; yeasts (*Saccharomyces* spp) and *Actinomycetes* (*Strptomyces* 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). When effective micro-organisms cultures are applied to the soil they stimulate the decomposition of organic wastes and residues thereby releasing inorganic nutrients for plant uptake. Majority of the scientists who are engaged in

promoting this technology have no doubt that plant growth is just as good or batter and quality of plant products is superior to conventional farming (Bajwa et al., 1999a; Iwaishi, 2000; Xu et al., 2000; Javaid, 2006). However, experiences of some workers revealed that the effect of effective microorganisms on crop yield was usually not evident or even negative particularly in the first test crop (Javaid et al., 2008). It is often difficult to establish the predominance of effective microorganisms cultures in soil with only a single application and during only one season. Certain soil properties and the indigenous soil microbial populations are often constraints to the establishment of these microorganisms (Bajwa et al., 1995; Javaid et al., 1997). Black gram is a grain legume widely cultivated in Pakistan, India and other Asian countries. It is part of diet for millions of people in these countries and a cheap source of protein with 17 - 34% of protein in seeds (Gour, 1993). An important feature of the mashbean plant is its ability to establish a symbiotic partnership with specific bacteria, setting up the biological N2-fixation process in root nodules by rhizobia that may supply the plant's needs for N (Mahmood and Athar, 2008; Mandal et al., 2009). The present study was carried out to investigate the effect of two *Bradyrhizobium japonicum* strains; TAL- 102 (soybean isolate) and MN-S (mungbean isolate) on growth, nodulation and yield of mashbean and role of EM in improving the efficacy of these strains in different soil amendment systems.

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

This experiment was a continuation of a previous experiment where mungbean [*Vigna radiata* (L.) Wilczek] was cultivated. Experiment was conducted in earthen pots of 20 cm diameter and 30 cm deep. The pot soil was amended either with farmyard manure (FYM) @ 5 g/100 g, *Trifolium alexandrianum* green manure (GM) @ 4 g/100 g (on dry weight basis), NPK fertilizers or left unamended. A basal dose of 20 mg kg-1 N as urea, 30 mg kg-1 P2O5 as triple supper phosphate and 30 mg kg-1 K2O as potassium sulphate was supplied to the NPK amended pot soil. Pots were irrigated with tap water of good quality and left for 15 days for decomposition of organic matter. Mungbean was sown in these pots. After harvesting the mungbean crop, the present study was conducted in the same pots. No more GM and FYM were added for the present study. However, NPK fertilizers were added to the respective pots at the same rate as was for mungbean mentioned above.

Procurement of *B. japonicum* and EM

Two peat based *B. japonicum* inocula namely *B. japonicum* st. TAL- 102 and *B. japonicum* st. MN-S were obtained from Nuclear Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan. *B. japonicum* st. TAL- 102 is an exotic strain originally isolated from soybean while *B. japonicum* st. MN-S is a local strain isolated from mungbean.

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 ´ 1011 cfu ml^{-1}, photosynthetic bacteria at 1´106 cfu ml^{-1} and yeast 1 ´ 103 cfu ml^{-1} of suspension (Higa, 2000). The EM stock solution was diluted by adding water in the ratio of 1:1000. Respective pots of EM treatments were irrigated with 500 ml of dilute solution of EM (1:1000) 15 days prior to mungbean sowing in the previous experiment. These pots also received 500 mL of dilute EM solution at fortnight intervals throughout the experimental period for mungbean (previous experiment) as well as for black grams (present experiment).

Treatments and experimental design

There were 6 treatments in each of the 4 soil amendment systems. These include:

i) Control (without any microbial inoculation).
ii) Effective Microorganisms (EM).
iii) *B. japonicum* st. MN-S.
iv) *B. japonicum* st. MN-S + EM.
v) *B. japonicum* st. TAL- 102.
vi) *B. japonicum* st. TAL- 102 + EM.

Black gram seeds were surface sterilized with 1.0% sodium hypochlorite solution followed by several washings with sterilized water. Seeds were soaked in sterilized water for 2 h and left in covered petri plates over night to facilitate rapid and uniform germination.

Seeds for respective *B. japonicum* treatments were pelted with peat based single strain inocula of *B. japonicum* st. TAL- 102 and *B. japonicum* st. MN-S with concentrated sugar solution as an adhesive. Initially 4 seeds were sown in each pot, which were thinned to 2 uniform seedlings on emergence. Each treatment was replicated 3 times. Pots were arranged in a completely randomized design on a bench in a wire netting house under natural conditions of light and temperature. Plants were irrigated with tap water of good quality whenever required.

Data collection and statistical analysis

Plants were harvested at flowering and maturity stages. The data regarding shoot length, root and shoot biomass were recorded at both the harvesting stages while that of number and biomass of nodules were recorded only at flowering stage. Data regarding various yield parameters; pod number, pod length, number of seeds per pod and grain yield were recorded at maturity. 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

Effect of microbial inoculation on shoot and root growth

Analysis of variance shows that effect of *B. japonicum* (B) inoculation was significant for shoot length (Table 1). At flowering stage, effect of either of the two *B. japonicum* strains was not much pronounced. However, at maturity stage, both the *B. japonicum* strains markedly enhanced shoot length in un-amended and farmyard manure (FYM) amended soils (Table 2). Both the *B. japonicum* strains enhanced shoot biomass in NPK amended soil. Effect of *B. japonicum* st. MN-S was more pronounced and significant both at flowering stage and maturity (Table 2). Effect of *B. japonicum* inoculation was also significant for root biomass (Table 1). In green manure (GM) amended soil, both the *B. japonicum* strains markedly suppressed root biomass at maturity stage. By contrast, in NPK amended soil a significant increase in root biomass was recorded by inoculation of either of the two *B. japonicum* strains. In un-amended as well as in FYM amended soil, neither of the two *B. japonicum* strains exhibited pronounced effect on root biomass (Table 2). Analysis of variance reveals the significant effect of EM application on shoot length and biomass as well as on root biomass. The interactive effect of EM and soil amendment (A) was also significant for shoot length. Similarly, the effect of A ´ B ´ EM was significant for root biomass (Table 1). The most pronounced and significant effect of EM application was observed on shoot dry biomass in NPK amendment. Similar effect of EM application on root dry biomass was also recorded in NPK amendment at maturity stage. Neither of the two *B. japonicum* strains showed significant response to EM application with respect to root and shoot growth in any of the 4 soil amendment systems (Table 2).

Table 1. ANOVA for the effect of growth stage, soil amendments, *B. japonicum* and effective microorganisms (EM) on root and shoot growth of *V. mungo*.

Sources of variation	df	Mean Squares		
		Shoot length	Shoot biomass	Root Biomass
Treatments	47	87**	4.4**	0.35**
Growth stage (G)	1	165**	1.32 ns	1.35**
Soil Amendments (A)	3	954**	48.97**	20.45**
***B. japonicum* strains (B)**	2	73*	1.40ns	0.45**
EM	1	115*	10.79**	1.60**
G ´ A	3	9.81ns	4.81**	0.09**
G ´ B	2	13.7ns	1.01ns	0.02ns
G ´ EM	6	0.34ns	0.012ns	0.0005ns
A ´ B	6	11.1ns	1.13ns	0.012ns
A ´ EM	1	92.1**	0.07ns	0/013ns
B ´ EM	3	10.4ns	1.24ns	0.007ns
G ´ A ´ B	3	22.6ns	0.95ns	0.004ns
G ´ A ´ EM	2	10.4ns	0.29ns	0.007ns
G ´ B ´ EM	2	17.3ns	0.30ns	0.02ns
A ´ B ´ EM	6	19ns	1.60ns	0.03*
G ´ A ´ B´ EM	6	5.3ns	0.42ns	0.04*
Error	96	15	0.65	
Total	**143**			

Table 2. Effect of *B. japonicum* and EM application on plant vegetative growth in *V. mungo*.

Treatments	Flowering Stage			Maturity Stage		
	Shoot Length (cm)	Shoot Dry Weight (g)	Root Dry Weight (g)	Shoot Length (cm)	Shoot Dry Weight (g)	Root Dry Weight (g)
Un-amended soil						
Control	33 a-g	3.6 b-f	0.52 ab	31 c-g	2.9 e-h	0.53 b-e
EM	37 a-c	4.4 a-c	0.53 ab	40 ab	3.7 c-f	0.70 ab
B. japanicum st. MN –S	35 a-e	4.6 a-c	0.64 a	38 a-c	3.6 d-f	0.60 b-e
B. japanicum st. MN-S +EM	39 a	5.0 ab	0.54 ab	40 ab	3.6 d-f	0.63 bc
B. japanicum st. TAL -102	35 a-e	4.1 a-d	0.46 b-d	37 a-d	3.0 e-h	0.64 bc
B.japanicum st. TAL-102+EM	38 a b	4.0 a-e	0.50 a-c	40 ab	4.6 a-e	0.54 b-e
Farmyard Manure						
Control	32 a-g	2.2 g-i	0.14 g	34 b-e	3.5 d-g	0.22 g
EM	29 d-i	2.7 d-i	0.20 e-g	30 d-h	3.5 d-g	0.30 f-i
B. japonicum st. MN-S	30 c-h	1.7 h-i	0.16 fg	40 ab	3.1 e-h	0.20 h-j
B. japonicum st. MN-S + EM	32 b-g	2.24 f-i	0.21 e-g	36 a-e	3.7 c-f	0.38e-h
B. japonicum st. TAL- 102	35 a-d	2.8 d-h	0.21 e-g	38 a-c	2.9 e-h	0.25 g-j
B. japonicum st. TAL- 102 + EM	36 a-c	3.5 c-g	0.32 d-f	34 b-e	3.6 d-f	0.44c-g
Green Manure						
Control	24 h-j	1.3 i	29 d-h	2.3 f-i	0.19h-j	0.20 i
EM	22 j	1.55 hi	26 f-h	1.9 g-i	0.19 h-j	0.40 f-i
B. japonicum st. MN-S	23 ij	2.0 hi	25g-h	1.3i	0.09 i-j	0.43 f-j
B. japonicum st. MN-S + EM	29 f-j	2.2 g-i	29 d-h	2.3 f-i	0.14 ij	0.23g-h
B. japonicum st. TAL- 102	29 e-j	1.5 hi	23 h	1.3 i	0.08j	0.34 f-j
B. japonicum st. TAL- 102 + EM	26 g-j	2.6 e-I	26 f-h	1.8 hi	0.13ij	0.54 c-h
NPK Fertilizers						
Control	34 a-f	2.8 d-i	0.34 c-e	33 b-f	3.6 d-f	0 .46 c-f
EM	35 a-f	4.4 a-c	0.42 b-d	39 ab	5.7 a	0.90 a
B. japonicum st. MN-S	33 a-f	5.4 a	0.35 c-e	36 a-e	5.4 b	0.72 b
B. japonicum st. MN-S + EM	38 ab	4.5 a-c	0.46 b-d	40 ab	4.9 a-d	0.57 b-e
B. japonicum st. TAL- 102	33 a-g	3.7 b-f	0.34 a	36 a-e	4.1 b-e	0.69 ab
B. japonicum st. TAL- 102 + EM	37 a-c	4.2 a-d	0.55 ns	43 a	5.3 a-c	0.57 b-d

Effect of microbial inoculation on nodulation

Effect of soil amendments was significant both for number and fresh biomass of nodules (Table 3). The highest nodules number was recorded in un-amended soil followed by NPK amendment. Both the organic amendments resulted in a marked suppression in nodules number. Adverse effect was more pronounced due to GM than FYM amendment (Table 4). *B. japonicum* inoculation showed a significant effect on nodulation. Nodules number was significantly enhanced by both the *B. japonicum* strains in un-amended as well as in NPK amended soil. *B. japonicum* st. MN-S was more effective in un-amended soil while st. TAL-102 was more effective in NPK amendment. Effect of inoculation on nodules biomass was also much pronounced in these two types of soils. Conversely, inoculation of either of the two *B. japonicum* strains failed to show significant effect on number and biomass of nodules in GM and FYM amended soils (Table 4). Effect of EM application on nodulation was variable with respect to soil amendments.

Analysis of variance shows that the interactive effect of EM and soil amendments was highly significant (P _ 0.01 and 0.001) both for nodule number and biomass (Table 3). In FYM amended soil, EM application markedly enhanced nodule number both in *B. japonicum* inoculated and un-inoculated treatments. In contrast to that, in NPK amendment, EM application suppressed number as well as biomass of nodules in *B. japonicum* inoculated plants. In other soil amendment systems, effect of EM application on nodulation was not much pronounced (Table 4).

Table 3. ANOVA for the effect of soil amendments, *B. japonicum* and effective microorganisms (EM) on nodulation and yield characteristics of *V. mungo*.

Trait	df	Mean Squares					
		Nodule No.	Nodule Fresh Weight	Pod No	Pod Length	Seed/Pod	Grain Yield
Treatments	23	8190	0.14***	58***	0.15***	0.95***	2.0***
Soil amendments (A)	3	48708	0.723***	296***	0.63**	4.26***	11.9***
B.japonicum strains (B)	2	4629	0.063*	4.5 ns	0.11 ns	0.44 ns	0.019 ns
EM	1	15 ns	0.030 ns	40.5 ns	0.051ns	0.015 ns	0.809 ns
A ×B	6	1575ns	0.028 ns	32.4 ns	0.059ns	0.089 ns	0.753*
A× EM	3	4444**	0.195***	22.6 ns	0.033ns	0.617 ns	0.75 ns
B ×EM	2	1312ns	0.068*	24.0 ns	0.107ns	1.498**	0.071 ns
A ×B ×EM	6	1262	0.014 ns	12.5 ns	0.095ns	0.466 ns	0.536 ns
Error	48	901	0.02	15.8	0.065	0.332	0.327
Total	**71**						

Effect of microbial inoculation on yield

Analysis of variance shows that effect of soil amendments was significant for various yield parameters; number of pods per plants, pod length, number of seeds per pod and grain yield (Table 3). Generally, values of these parameters were lower in GM amendment as compared to other soil amendment systems (Table 4). *B. japonicum* st. MN-S enhanced number of pods per plant by 26% in un-amended soil. By contrast, both the species reduced pod number by 40% in GM amendment. However, all the effects were insignificant statistically. Effect of *B. japonicum* inoculation on pod length and number of seeds per pod was also insignificant in all the 4 soil amendment systems. Grain yield was significantly enhanced by 46% due to each of the two *B. japonicum* strains in NPK amended soil. EM application resulted in significant increase of 48% in grain yield in NPK amended soil. In general, effect of EM application on various yield parameters was insignificant (Tables 3 and 4).

Table 4. Effect of *B. japonicum* and EM application on nodulation and yield of *V. mungo*.

Treatments	Nodule No.	Nodule Fresh Wt (g)	Pod No.	Pod Length (cm)	Seed / Pod	Grain Yield (g)
Un-amended soil						
Control	116 f 0.	38 c-g	17 a-f	3.75 a	5 ab	2.8 a
EM	97 c-f	0.45 b-f	18 a-e	3.64 a	5 ab	2.9 a
B. japonicum st. MN-S	175 ab	0.54 a-d	23 a	3.47 a	4 a-d	3.1 a
B. japonicum st. MN-S + EM	188 a	0.73 a	16 a-f	3.51a	4 a-d	2.3 ab
B. japonicum st. TAL-102	158 ac	0.61 a-d	16 a-f	3.60 a	5 ab	2.3 ab
B. japonicum st. TAL-102 + EM	142 a-e	0.55 a-d	16 a-f	3.61 a	4 a-d	2.2 a-c

Farmyard Manure						
Control	76 f-i	0.14 gh	12 c-g	3.4 a	4.4 a-c	1.4 b-d
EM	106 b-f	0.18 gh	12 c-g	3.4 a	4.3 a-d	1.2 c-d
B. japonicum st. MN-S	72 f-i	0.09 h	11 d-g	3.2 a	3.6 b-e	0.9 d
B. japonicum st. MN-S + EM	123 a-f	0.24 e-h	10 e-g	3.3 a	4.2 a-d	1.2 cd
B. japonicum st. TAL-102	82 e-h	0.18 gh	13 b-g	3.4 a	4.0 b-e	1.5 b-d
B. japonicum st. TAL-102+*EM*	116 b-f	0.16 gh	20 ab	3.5 a	4.1 a-d	2.2 a-c
Green Manure						
Control	19 i	0.55 a-d	10 e-g	3.4 a	3.2 de	1.0 d
EM	25 hi	0.66 ab	10 e-g	3.2 a	3.6 b-e	1.1 d
B. japonicum st. MN-S	19i	0.50 a-e	6 g	2.7 a	2.8 e	0.6 d
B. japonicum st. MN-S + EM	30 g-i	0.60 a-d	10 e-g	3.3 a	3.7 b-e	1.2 cd
B. japonicum st. TAL-102	22 hi	0.70 ab	6 g	3.1 a	3.4 c-e	0.6 d
B. japonicum st. TAL-102 + EM	19 i	0.65 a-c	10 e-g	3.1 a	3.4 c-e	1.0 d
NPK Fertilizers						
Control	87 d-g	0.38 d-g	13 b-g	3.2 a	4.1 a-d	1.4 d
EM	109 b-f	0.24 e-h	16 a-f	3.5 a	4.6 ab	2.7 a
B. japonicum st. MN-S	149 a-d	0.60 a-d	17 a-f	3.4 a	4.3 a-c	2.6 a
B. japonicum st. MN-S + EM	100 b-f	0.24 e-h	20 a-c	3.5 a	4.3 a-c	2.9 a
B. japonicum st. TAL-102	163 ab	0.76 b-f	11 d-g	3.7 a	5.1a	2.6 a
B. japonicum st. TAL-102 + EM	78 f-I	0.20 f-h	18 a-d	3.3 a	3.4 c-e	2.4 a

Discussion

In the present study, suitability of cross inoculation of two *B. japonicum* strains; TAL-102 (soybean isolate) and MNS (mungbean isolate) inoculation to mashbean for bather growth, yield and nodulation characteristics was studied in different soil amendment systems. Both the strains proved suitable for mashbean. However, the affectivity of the two inoculated strains was associated with the type of soil amendment. Nodule number was significantly increased by inoculation of either of the two strains in NPK amendment. Nodule biomass was also markedly enhanced in inoculated treatments in this soil amendment system. As a consequence of improved nodulation, a similar significant improvement in grain yield was also evident due to inoculated *B. japonicum* strains. Earlier, Dubey (1998) obtained highest grain yield in soybean when host plant was inoculated with *Bradyrhizobium* in combination with NPK fertilizers. In the present study, response of nodulation to *B. japonicum* strains inoculation in un-amended soil was similar to that of response in NPK fertilizers amendments. However, unlike that of NPK fertilizer amendment, grain yield was not enhanced in unamended soil in response to *B. japonicum* inoculation.

Earlier, Mahmood and Athar (2008) reported that cross inoculation of mashbean with rhizobia isolated from *Dalbergia sissoo*, *Leucaena leucocephala*, *Pithecellobium dulce*, *Prosopis cineraria*, *Prosopis glandulosa* and *Prosopis juliflora* significantly enhanced dry weight and nitrogen contents of mashbean. In the present study, in both the organic matter amendments; farmyard and green manure amended soils, nodulation was very poor as compared to NPK amended and un-amended soils. Inoculation of both the *B. japonicum* strains failed to enhance nodulation in these two organic matters amended soils. Effect of EM application on nodulation was variable in different soil amendment systems. EM application markedly enhanced nodule number both in *B. japonicum* inoculated and un-inoculated treatments in FYM amended soil. Conversely, in NPK amendment, EM application adversely affected the nodulation both in terms of number and biomass.

In GM amendment as well as in unamended soil, effect of EM application was not pronounced. Earlier reports regarding the effect of EM application on nodulation are also contradictory. EM application caused a significant reduction in nodule number but increased the size and biomass of nodules in *Trifolium alexand rinum* (Bajwa et al., 1999b). By contrast, Javaid et al. (2000) noted a significant increase in nodulation in *Vigna radiata* due to EM application. Javaid et al. (2002) have reported similar effects of long-term EM application and organic manures on nodulation in *Phaseolus vulgaris* L. In seems probable that soil amendments as well as indigenous population of soil microorganisms determine the nodulation response of host plant to EM application. Similar to that of nodulation, effect of EM application on plant growth and yield was also variable in different soil amendments. The most pronounced and significant effect of EM application on shoot and root dry biomass was observed in NPK amendment. Likewise, EM application resulted in 48% increase in grain yield in NPK amended soil without *B. japonicum* inoculation. In rest of the treatments, effect of EM application was not much pronounced. Earlier there are contradictory 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., 1995, 1999b; Diass et al., 2008; Javaid 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., 2000, 2002). 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 previous studies. 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 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. However, the present study reveals that the effect of EM application on crop growth and yield is associated with the type of soil amendment used. This study concludes that the benefits of *B. japonicum* strains TAL- 102 and MN-S and EM to black gram can be best exploited by applying these biofertilizers in NPK amended soils. However, further field trials are required before these findings are recommended to the farmers for field application of these biofertlizers.

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