

Effect of EM on Nitrogen Fixation by Bush Bean and Mungbean

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Abstract

The effect of effective microorganisms (EM) on nodulation parameters and yields of bush bean and mungbean on soils with low and high populations of rhizobia were evaluated. Two soils were selected because of the adverse effects of high temperatures and dry conditions of the tropics on growth and maintenance of inherent populations of beneficial soil microorganisms. The experimental treatments included the use of fertilizer, organic amendments, and a control, with and without application of effective microorganisms. Estimates were made on the nodulating capacity, nitrogenase activity, and growth and yield of plants for each treatment.

Application of effective microorganisms significantly increased the most probable number counts of bacteria in soils. The greatest change was observed in soil with low inherent microbial populations. Nodulation and nitrogenase activity, characteristics of both legumes, were significantly enhanced, especially when grown in nutrient depleted soil. In addition, the beneficial impact was most prominent with bush beans which generally do not fix significant quantities of atmospheric nitrogen infertile and biologically active soils.

Addition of fertilizer decreased the process of biological nitrogen fixation. However, this adverse impact was reduced with addition of EM. In contrast, application of nitrogen-rich organic materials had a significant beneficial impact on the process of biological nitrogen fixation, especially when applied with EM. The use of organic materials with a high C:N ratio did not increase the rates of nitrogen fixation, especially in the absence of effective microorganisms.

Introduction

Food legumes are an important component of farming systems in the developing world, both ecologically and nutritionally. Although considered secondary to cereals, they are grown over a wide range of environments. Furthermore, they are generally planted on marginal soils unsuited for cereals or other major food and industrial crops (APO, 1982; Singh, 1985).

The two principal properties that make legumes useful to agriculture are their high protein content and their ability to fix atmospheric nitrogen (Beck and Roughley, 1987). The benefit of biological nitrogen fixation is realized only if symbiosis of the plant with the bacteria operates efficiently. The factors determining the symbiotic process are the genetic constitution of the host plant and bacteria, environment, and technological inputs such as inoculum, fertilizers and pesticides (Munns, 1977).

Tropical soils have a diverse range of physical, chemical and biological properties. These affect biological nitrogen fixation by their impacts on rhizobial populations. Thus, the ineffectiveness of nitrogen fixation in tropical soils can, in most instances, be attributed to the lack of suitable bacteria (Date, 1988), and can be overcome by the use of inocula to increase the rhizobial populations (Graham, 1981).

Inocula are not widely used in the developing world and, therefore, the process of biological nitrogen fixation is largely ineffective in tropical soils. The high cost of production, the requirement for controlled storage facilities, the absence of a sound infrastructure for marketing and distribution of biological products that are easily damaged by high temperatures, and the lack of knowledge about their proper use (Beck and Roughley, 1987) all contributed to the non-use of inocula. Recent research in Japan (Higa, 1988) illustrates the benefits of using effective microorganisms (EM) to increase yields of crops under a wide range of conditions. EM is a microbial inoculant that contains a wide range of beneficial bacteria, yeast and fungi and is reported to increase the effective microflora in soils and to transform marginal soils into acceptable biodynamic systems which can produce suitable environments for plant growth (Higa, 1988).

An evaluation of the efficiency of EM cultures for improving biological nitrogen fixation of two

uninoculated tropical food legumes, namely bush beans or vegetable beans (*Phaseolus vulgaris*) and mungbean (*Vigna radiata*), was the basis of this investigation. The legumes were selected on the basis of their nodulation characteristics; mungbean and bush beans are species with high and low nitrogen-fixing abilities, respectively (Lawn and Ahn, 1985; Graham, 1981). Two soils which had been either cultivated or non-cultivated with legumes for the last three years were selected for this study.

Materials and Methods

The experiment was conducted as a greenhouse trial in the Faculty of Agriculture, University of Peradeniya, Sri Lanka. The environmental conditions for the experimental period were: rain-fall, 758 mm; mean daily temperature, $27.5 \pm 1.6^\circ\text{C}$; mean daily relative humidity, $79.45 \pm 3.25\%$; and day length, 9 to 10 hours.

The soils were obtained from the University farm, 20 km from the campus. Soil A had been cultivated with legumes for the past three years, while Soil B had no history of legume cultivation during the same period. These soils were selected after screening for physical, chemical and biological properties (Table 1) to characterize differences in rhizobial populations (Somasegaram and Hobson, 1985) using *Vigna unguiculata* (cowpea) as the test crop.

Table 1. Characteristics of the Experimental Soils.

Property	Soil A	Soil B
Physical Properties		
Soil type	Ultisol	Ultisol
Texture	Sandy clay loam	Sandy loam
WHC (%)	24.6	18.5
Chemical Properties		
pH (1:2 H ₂ O)	6.4	6.8
Organic matter (%)	1.4	1.0
N (%)	0.24	0.11
P (ppm)	34.2	25.9
CEC (meq/100 g soil)	32.0	24.2
K (meq/100 g soil)	0.91	0.56
Biological Properties		
Most probable number of cowpea rhizobia (cells/g soil)	1.04×10^3	84

The soils were passed through a 5-mm screen and approximately 4 kg of each soil was put into individual plastic pots. The following treatments were imposed on the soils.

- A. Organic material with different C:N ratios was incorporated at a rate equivalent to 5 mt of fresh material per ha two weeks before planting. The organic matter sources were coir dust (C:N ratio, 58.4) and *Giliricidia* leaves (C:N ratio, 14. 1)
- B. Organic materials plus 5 ml of a suspension of effective microorganisms (EM 4) per pot prepared by the method of Higa (1989 Personal Communication) were incorporated 2 weeks before and after planting.
- C. EM 4 was added to the soil at similar times without organic materials.
- D. Inorganic fertilizers (equivalent to 25 kg N, 80 kg P₂O₅ and 100 kg K₂O per ha) incorporated into the soil at planting.
- E. Inorganic fertilizers with 5 ml of EM 4 applied two weeks before and after planting.
- F. A control treatment without EM 4, organic materials, or fertilizer.

The treatments were arranged in a Completely Randomized Block Design with four replicates for each crop. Two seeds per pot (germination 91 ± 2.3 percent) were planted two weeks after the addition of organic matter and EM 4 for each species. The plants were watered regularly; and weeds

were removed once by hand. No pesticides were used.

The following data were collected for each treatment:

- 1) Establishment of the crop three weeks after planting.
- 2) At 50 percent flowering, four plants per treatment per replicate were uprooted carefully. Nodule numbers, fresh weight and activity by acetylene reduction assay (ARA) (Hardy *et al.*, 1968) were determined.
- 3) At maturity, pod yields of bush bean and mungbean and seed yields (14 percent moisture) were determined for 8 plants per treatment per replicate.
- 4) After the final harvest, Rhizobium most probable number (MPN) counts on soils for different treatments were determined by the method of Somasegaram and Hobson (1985) using *Vigna unguiculata* (cowpea) as the test crop.

The data were subjected to statistical analysis as described by Gomez and Gomez (1983).

Results and Discussion

The soils selected for the experiment had different physical, chemical and biological properties (Table 1). Soil B had a greater percentage of sand, thus a lower water holding capacity. Soil A had higher organic matter and nutrient content than Soil B. Thus, soil A could be considered more suitable for food crop production. Evaluation of microbial populations also illustrated a significantly greater number of rhizobia associated with cowpea in soil A. This result suggests that the cultivation of legumes tends to maintain rhizobial populations in soils.

The impact of the treatments on nodulation characteristics of vegetable beans and mungbean at the 50 percent flowering stage are presented in Table 2. Vegetable beans had lower nodule numbers and weights than mungbean in all treatments. This confirms the poor nodulation characteristics of vegetable beans as reported by Graham (1981). The promiscuity of mungbean in terms of nodulation (Lawn and Ahn, 1985) was also shown in this study. Soil A induced better nodulation in both crops irrespective of the treatment. This result suggests that in soils with low rhizobial populations inoculation may be an important management tool to increase nodulation in common legume species.

Incorporation of organic matter affects the natural rhizobial populations (Odu, 1977) because of changes in nutrient and carbohydrate levels in the soil. Addition of coir dust with a high C:N ratio reduced nodule numbers of both species; this reduction was greater in the soil with low rhizobial populations. However, addition of *Gliricidia* leaves with a low C:N ratio had no significant impact on nodule numbers of either species. This suggests the beneficial effect of incorporating organic materials with low C:N ratios, which ensures the availability of nutrients for microbial decomposition. In contrast, organic materials with high C:N ratios can lead to a temporary depletion of available nutrients during decomposition which, in turn, can adversely affect nodulation.

Application of EM cultures significantly increased the nodulation of both species. However, the impact is most evident for vegetable bean, the poor-nodulating species, when planted in soil B with a low rhizobial population. This result illustrates the benefits derived from inoculation of poor-nodulating species, especially when they are planted in soils where legumes have not been previously cultivated. In addition, the data indicates the capacity of EM to act as an inoculant for legumes.

Addition of EM plus organic materials increased nodulation of both species, again illustrating the beneficial effects of these microorganisms. The trend was similar in both species, although the response by vegetable bean was greater than for mungbean. Also, the application of EM to *Gliricidia* leaves produced a greater response than when applied to coir dust.

Nitrogen fertilizers, especially when applied in high quantities, have a negative impact on nodulation in legumes (Munns, 1977). The data also showed that fertilizer N reduced nodulation; the adverse effect was greater in vegetable bean which had a lower rhizobial population.

Research (Date, 1988) has shown that inoculation had little impact in the presence of fertilizer, especially nitrogen. However, the application of EM with chemical fertilizer significantly increased

nodulation of selected legumes.

Nodule weights follow a trend similar to that of nodule numbers (Table 2). Comparison of percentage increase in the two parameters illustrated that changes in nodule weights are greater. This suggested that EM has the capacity to produce heavier nodules, which, in turn, can be considered as effective nitrogen fixing units.

Table 2. Nodulation of Vegetable Bean and Mungbean at 50 Percent Flowering as Affected by Treatments.

Treatment	Vegetable Bean				Mungbean			
	Soil A	SoilB	Soil A	SoilB	Soil A	SoilB	Soil A	SoilB
	Nodule Number/Plant		Nodule Wt. (mg)/Plant		Nodule Number/Plant		Nodule Wt. (mg)/Plant	
Organic matter 1	4	2	5.8	2.9	32	15	30.8	13.5
Organic matter 2	10	7	9.6	6.1	49	22	50.8	24.4
Organic matter 1 +EM	8	7	10.4	5.7	41	18	54.8	17.5
Organic matter 2 +EM	27	21	34.6	25.8	65	36	78.9	39.5
EM alone	23	20	27.4	22.1	56	30	66.5	32.6
Fertilizer	6	2	5.5	3.1	28	16	27.8	17.4
Fertilizer +EM	10	6	11.6	7.5	41	21	45.8	26.4
Control	12	6	9.8	5.6	48	25	42.7	22.8
LSD (P=0.05)	1.9	0.8	0.4	0.3	2.7	2.0	0.3	0.9

The impact of the treatments on nodule activity (Table 3) suggested a close relationship between nodulation parameters and acetylene reduction by microorganisms in the nodules. As with nodulation, organic matter with a high C:N ratio and inorganic fertilizer reduced nodule activity. However, EM has a beneficial effect on nodule activity when applied in combination with organic amendments or fertilizer, and it is useful in increasing nodulation and the nitrogen-fixing capacity of legumes.

Table 3. Nitrogenase Activity of Vegetable Bean and Mungbean at 50 Percent Flowering as Affected by Treatment.

Treatment	Vegetable Bean (μ mol /plant/hour)		Mungbean (μ mol /plant/hour)	
	Soil A	Soil B	Soil A	Soil B
Organic matter1	4.15	2.98	15.84	8.34
Organic matter2	14.84	7.24	28.06	17.06
Organic matter1 + EM	8.57	4.95	22.94	13.45
Organic matter2 + EM	28.06	13.99	60.41	32.42
EM alone	26.30	12.65	48.91	25.11
Fertilizer	5.78	3.12	14.69	8.85
Fertilizer + EM	8.80	5.16	22.98	14.26
Control	14.55	8.75	30.37	17.84
LSD (P=0.05)	3.22	1.86	5.71	2.01

Comparison of the percentage increase in nodule activity with EM had lower values than observed in nodule numbers and weights. This implies that EM increases nodulation rather than nodule activity. This phenomenon can be considered a result of the presence of a variety of beneficial microorganisms in EM (Higa, 1988) which in turn can enhance the process of nodulation rather than activity; it is observed especially when poor nodulating species are grown on soils with low populations of indigenous rhizobia.

Table 4 reports the effect of organic amendments, fertilizer and EM on the establishment and yield of the selected legumes. Establishment of both species was not significantly affected by any of the

treatments, thus clearly showing the lack of either harmful or beneficial effects of organic amendments, fertilizer or EM at very early stages of growth.

Yields of the two species were significantly affected by the treatments. Soils without additives produced low yields; vegetable bean grown in soil B showed the greatest treatment effect. Application of organic amendments increased the yield of both species. This was especially evident when *Gliricidia* leaves with a low C:N ratio were used as the organic amendment. Application of organic materials with a high C:N ratio tended to utilize (and immobilize) nutrients from the soil for decomposition, thus depriving the crop of available plant nutrients (Mengel and Kirkby, 1987).

Application of EM alone does not produce high yields. However, when added with organic material, yields of both species increased significantly. Again, the greater beneficial effect was observed with vegetable bean. This phenomenon can be attributed to the reported ability of EM to effectively decompose organic matter thus releasing nutrients for plant growth (Higa, 1988). Yield increases with EM were greater when the crops were planted after incorporation of organic materials with low C:N ratios. This indicated the ability of EM to further enhance yields of legumes grown with good quality organic amendments. Application of chemical fertilizer produced very high yields of both legumes, irrespective of the soil type. However, yields of plants grown in soil A, the more fertile soil, were greater. This was the result of the ability of chemical fertilizer to provide the growing plants with nutrients required in readily available form (Mengel and Kirkby, 1987). However, the application of EM along with fertilizer increased yields significantly (Table 4). This was attributed to the ability of EM to decompose organic materials in the soil, thereby releasing additional available nutrients for plant growth. The ability of EM to change the conditions of the rhizosphere to a zymogenic state, and thereby provide a more favorable environment for plant growth (Higa, 1988) could also be considered a causal factor which needs further study.

Table 4. Effect of Treatment on Plant Establishment and Yield Per Plant of Vegetable Bean and Mungbean.

Treatment	Vegetable Bean				Mungbean			
	Soil A	Soil B	Soil A	Soil B	Soil A	Soil B	Soil A	Soil B
	Establishment (%)		Yield (g)		Establishment (%)		Yield (g)	
Organic matter1	85	86	28.2	22.2	91	86	4.6	2.9
Organic matter2	88	89	35.1	26.1	89	88	6.9	5.2
Organic matter1 + EM	86	90	34.6	25.2	87	89	5.6	3.3
Organic matter2 + EM	88	87	45.5	35.2	94	89	8.0	7.0
EM alone	87	86	26.6	21.1	86	86	4.0	2.9
Fertilizer	85	87	74.6	60.8	98	86	12.1	8.6
Fertilizer + EM	86	85	77.9	64.1	91	88	13.0	9.1
Control	83	85	24.6	12.8	90	85	3.1	2.1
LSD (P=0.05)	6.9	5.2	10.8	5.0	6.0	4.3	0.4	0.2

The rhizobial populations of the soils before and after the experiment are shown in Table 5. While rhizobial populations declined in the absence of soil additives, with addition a high C:N organic materials (coir dust) or fertilizer, the application of EM increased the rhizobial population significantly, irrespective of the treatment or soil type. This suggests that the beneficial effects of EM are not restricted to plant factors alone, but may have the capacity to increase the numbers of rhizobia in the soil (Somasegaram and Hobson, 1985).

A comparison of microbial populations in the two soils shows that there was a greater percentage increase with soil B, a soil which initially had a very low number of rhizobia. The numbers of rhizobia indicated that the application of EM with good quality organic matter (e.g., *Gliricidia*) had the greatest effect, and may result in the ability of organic matter with a low C :N ratio to provide nutrients for the rhizobial population over a long period of time. Again, however, further work is required.

Table 5. Influence of Treatment on Rhizobial MPN Counts of Two Soils after 150 Days.

Treatment	Soil A	Soil B
Inception of trial	1.04 x 10 ³	86
Organic matter1	0.95 x 10 ³	85
Organic matter2	1.14 x 10 ³	98
Organic matter1 + EM	1.42 x 10 ³	152
Organic matter2 + EM	2.18 x 10 ³	266
EM alone	1.15 x 10 ³	95
Fertilizer	0.84 x 10 ³	81
Fertilizer + EM	1.86 x 10 ³	113
Control	0.94 x 10 ³	89
LSD (P=0.05)	104.2	5.7

Unit: (cells/g soil)

Conclusions

Successful cultivation of legumes is dependent on the availability of adequate rhizobia in the soil to fix atmospheric nitrogen (Beck and Rughley, 1987). However, all soils do not have significantly high populations of rhizobia, and seed inoculation is a common practice in tropical legume cultivation (Ayanaba, 1977). The inoculum is a biological product and its successful use in the tropics depends on the survival of the organisms during transportation, and before and soon after sowing. Although the inoculum is available in small packets, its efficacy may be low because of poor shelf-life and the adverse effect of high temperatures. Careful application for successful infection is generally limited to the time of planting (Somasegaram and Hobson, 1985). Effective microorganisms (EM) are generally available in liquid suspensions and are not affected by humidity, although relatively cool storage conditions are required. Refrigeration is not essential for maintaining microbial populations. EM can also be applied to the soil before planting and at later stages of growth, thereby avoiding the need for seed inoculation.

The effect of EM in improving nodulation, nitrogenase activity, and yield of two common tropical food legumes is seen in this study. The results indicate the usefulness of EM application with organic amendments and chemical fertilizer, especially in poor soils with low rhizobial populations. EM solutions are effective inocula, which can be easily used. The ability of EM to enhance organic matter decomposition (Higa, 1988) tends to make the microorganisms more versatile as biological agents for legume production. The study also revealed the ability of EM to increase rhizobial populations in soils even after the harvest of a legume crop. Further studies are needed in order to quantify the usefulness of EM in tropical legume production.

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