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Enhancing crop growth, nutrients availability, economics and beneficial rhizosphere microflora through organic and biofertilizers

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Abstract - Field experiment was conducted on fodder maize to explore the potential of integrated use of chemical, organic and biofertilizers for improving maize growth, beneficial microflora in the rhizosphere and the economic returns. The treatments were designed to make comparison of NPK fertilizer with different combinations of half dose of NP with organic and biofertilizers viz. biological potassium fertilizer (BPF), Biopower, effective microorganisms (EM) and green force compost (GFC). Data reflected maximum crop growth in terms of plant height, leaf area and fresh biomass with the treatment of full NPK; and it was followed by BPF+full NP. The highest uptake of NPK nutrients by crop was recorded as: N under half NP+Biopower; P in BPF+full NP; and K from full NPK. The rhizosphere microflora enumeration revealed that Biopower+EM applied along with half dose of GFC soil conditioner (SC) or NP fertilizer gave the highest count of N-fixing bacteria (*Azotobacter, Azospirillum, Azoarcus* and *Zoogloea)*. Regarding the P-solubilizing bacteria, *Bacillus* was having maximum population with Biopower+BPF+half NP, and *Pseudomonas* under Biopower+EM+half NP treatment. It was concluded that integration of half dose of NP fertilizer with Biopower+BPF / EM can give similar crop yield as with full rate of NP fertilizer; and through reduced use of fertilizers the production cost is minimized and the net return maximized. However, the integration of half dose of NP fertilizer with biofertilizers and compost did not give maize fodder growth and yield comparable to that from full dose of NPK fertilizers.

Key words: N-fixation, diazotrophs, P-solubilizing bacteria, nutrients uptake, economics, biofertilizers.

INTRODUCTION

Maize (*Zea mays* L.) is the most important cereal fodder and grain crop under both irrigated and rainfed agricultural systems in the semi-arid and arid tropics (Hussan *et al.,* 2003). Green fodder has an immense importance for animal production and development. At present for 21 million heads of livestock in Pakistan, only 51% feed requirements of these animals can be met from green fodder. There is still a shortage of 26 million tonne of total digestible nutrients, and in terms of digestible protein shortage is 1.58 million tonne. So, there is a need of 150 million tonne green fodder to feed the animals and meet their nutrients requirement (Haqqani *et al.,* 2003).

Traditional use of chemicals fertilizers in agricultural production cannot be over-emphasized, but with fertilizer costs going up, these need to be supplemented or substituted with available organic or biofertilizers. Thus integrated plant nutrients system was introduced which includes application of organic and biofertilizers to supplement chemical fertilizers to maintain and increase the soil fertility for sustaining increased crop production. It helps for making the highly productive cereals mono cropping systems more sustainable (Rajendra *et al.,* 1998).

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Organic materials hold great promise due to their local availability as a source of multiple nutrients and ability to improve soil characteristics (Khaliq *et al.,* 2006). The improvement of fertility and quality of soil, especially under low input agricultural systems, requires the input of organic materials (Soumare *et al*., 2003; Naureen *et al*., 2005).

Biofertilizers contain different types of microorganisms, which have an ability to convert nutritionally important elements from unavailable to available form through biological processes (Hedge *et al*., 1999; Vessey, 2003). They have emerged as an important component of the integrated nutrient supply system and hold a great promise to improve crop yields. Biofertilizers containing N-fixer (*Azotobacter chroococcum*), P-solubilizer (*Bacillus megaterium*) and K-solubilizer (*Bacillus mucilaginous*) and arbuscular mycorrhizal fungi (*Glomus mosseae* and *Glomus intraradices*) have been developed (Hafeez *et al*., 2002; Wu *et al.,* 2005). The P-solubilizing microorganisms (PSM) render insoluble phosphate into soluble form through the process of acidification, chelation and exchange reactions (Gull *et al*., 2004; Son *et al.,* 2006). Therefore, many researchers have tried to increase the plant-available phosphate-fraction by means of PSM such as *Bacillus* (Raj *et al*., 1981), *Enterobacter* (Laheurte and Berthelin, 1988), *Pseudomonas* (Suh *et al*., 1995a, 1995b), *Agrobacterium*, *Aspergillus* (Varsha and Patel, 2000; Hameed *et al*., 2004) and *Pantoea agglomerans* (Son *et al.,* 2006).

The product of EM (effective microorganisms) contains selected species of microbes including predominant populations of lactic acid bacteria and yeasts, and smaller numbers of photosynthetic bacteria, actinomycetes and other types of organisms (Higa and Wididana, 1991). All of these are mutually compatible with one another and can coexist in liquid culture (Higa and Parr, 1994). A latest study indicated that application of EM to cotton increased the efficiency of both organic and mineral nutrient sources (Khaliq *et al.,* 2006). Economic analysis suggested that the use of 1/2 mineral NPK with EM+OM (organic matter) saved the mineral N fertilizer by almost 50% compared to a system with only mineral NPK application.

Currently, the need is to get maximum output with minimum cost, which is possible only if chemical fertilizers are supplemented with organic and biofertilizers. The present study was, therefore, envisaged with the objective to evaluate the comparative effect of chemical, organic and biofertilizers on the growth, yield and nutrients uptake of maize fodder. The interactions among the microorganisms of different microbial products and fertilizers were also investigated.

MATERIALS AND METHODS

Comparative study of organic and biofertilizers with chemical fertilizer was carried out for maize fodder production under rainfed conditions at the research farm of University of Arid Agriculture, Rawalpindi, Pakistan during the summer season of year 2003. The biofertilizers used for seed dressing were: Biological Potassium Fertilizer (BPF, 10 kg ha^{-1}), Biopower (2.5 kg ha^{-1}) and Effective Microorganisms (EM, 2.5 L ha^{-1}). While the organic fertilizers were Green Force Compost (GFC) enriched with NPK viz. GFC (NPK, 250 kg ha^{-1}) and Green Force Compost as soil conditioner GFC (SC, 500 kg ha^{-1}). The nature and composition of these experimental materials is given under:
BPF is a biofertilizer inoculum containing K-solubilizing bacteria *Bacillus mucilaginous*.
Biopower is a biofertilizer containing four N-fixing bacteria viz. *Azotobacter, Azospirillum, Azoarcus* and *Zoogloea*.
EM is a mixed inoculum containing mainly *Lactobacillus, Rhodopseudomonas, Actinomycetes* and yeast.
GFC (NPK) is composted municipal waste enriched with NPK fertilizer to have 15-15-15 percent NPK contents. Green Force Compost (GFC) is a commercial product.
GFC (SC) is composted municipal waste having NPK contents as 3.0-1.2-1.8 percent. This GFC is a soil conditioner (SC) product.
The experiment was laid out in a randomized complete block design with four replications. Urea, single super phosphate and sulphate of potash were used as source of NPK, respectively. All the fertilizers were applied in the field before maize sowing. Soil mixed with EM (10 L ha^{-1}) was broadcasted in T_7, T_8, and T_{12}. The experiment consisted of the following treatments:

- T_1 Full dose of NP fertilizers, 120-90 kg ha^{-1}, respectively
- T_2 Full dose of NPK fertilizers, 120-90-60 kg ha^{-1}, respectively
- T_3 Half dose of NP fertilizers, 60-45 kg ha^{-1}, respectively
- T_4 BPF as seed coating + full dose of NP fertilizer
- T_5 Biopower as seed coating + half dose of NP fertilizer
- T_6 Biopower + BPF + half dose of NP fertilizer
- T_7 EM (1% solution) for seed soaking, and broadcasted as soil mixed to 10 L EM with 100 kg soil ha^{-1} + half dose of NP fertilizer
- T_8 Biopower + EM as in T_7 + half dose of NP fertilizer
- T_9 Full dose of GFC (NPK), 250 kg ha^{-1}
- T_{10} Full dose of GFC (SC), 500 kg ha^{-1}
- T_{11} Half dose of GFC (SC), 250 kg ha^{-1} + Biopower as in T_5
- T_{12} Half dose of GFC (SC), 250 kg ha^{-1} + Biopower + EM as in T_7

Each treatment plot size was 7 m x 5 m, and maize variety Agaiti-85 (local name meaning the variety to be grown in early season) was grown as test crop. The seed was sown at the rate of 50 kg ha^{-1} with the help of hand drill on 1st August, 2004. The treatment effect was recorded on the growth parameters and fodder yield of crop at 60th day after sowing.

Physical and chemical properties of composite soil samples taken before sowing from the experimental field were determined. The soil was sandy loam in texture, had pH 7.3, electrical conductivity 0.30 dS m^{-1}, organic matter 0.43%, NO_3-N 1.2 mg kg^{-1}, available-P 5.2 mg kg^{-1}, and extractable-K 78.5 mg kg^{-1}. The plant samples taken at harvest were analysed for NPK contents and their uptake was calculated by employing the following formula:

$$\text{Nutrients uptake (kg ha}^{-1}\text{)} = \frac{\text{(Nutrient content in plants as \%) (Dry yield as kg ha}^{-1}\text{)}}{100}$$

Isolation of microorganisms. Rhizosphere microorganisms were isolated from each sample by serial dilution and spread plate method. One gram soil was dispersed in 10 mL of sterile distilled water and thoroughly shaken. One mL of above dilution was again transferred to 9 mL of sterile distilled water to form 10^{-2} dilution. Similarly, 10^{-3}, 10^{-4}, 10^{-5} dilutions were made for each soil sample. An aliquot of 0.1 mL from each dilution was taken with micropipette (0.01-0.1 mL range) and plated on agar medium specific for each microorganism to be isolated. After incubation total number of colonies formed was counted with the help of digital colony counter. Total count was determined as colony forming units (CFU):
CFU g^{-1} of moist soil = Mean plate count / (Volume of sample plated) (Dilution factor)
CFU g^{-1} of dry soil = CFU g^{-1} of moist soil (100 + percent moisture in sample / 100)

The following N_2-fixing and P-solubilizing bacteria mainly found in the rhizosphere were enumerated by using specific growth media for each species as suggested by authors quoted against each microorganism.
Azotobacter: Burk's medium (Cornish and Page, 2000) incubated at 26 °C for three days.
Azospirillum: Okon medium (Okon *et al*., 1976) incubated at 35 °C for 2 days.
Azoarcus: Jenson nitrogen free biotin medium (Döbereiner, 1995) incubated at 30 °C for three days until colour of media changed to blue then these were counted.
Zoogloea: Combined carbon medium formulated by Rennie (1981) incubated at 30 °C for 2 days.
Bacillus and *Pseudomonas:* Both these species of P-solubilizing bacteria were isolated on Pikovskaya's agar medium

(Pikovskaya, 1948) containing insoluble tricalcium phosphate. The plates were incubated at 26 °C for 7 days. After the growth, the colonies of *Bacillus* and *Pseudomonas* were isolated and re-cultured on nutrient agar media. The *Pseudomonas* was incubated at 20 °C for 24 hours, while *Bacillus* at 37 °C for 48 hours.

Purification of microorganisms. Colonies developed on each specified medium were picked with the help of sterilised inoculating wire loop and streaked on separate plate. All plates were then incubated at same temperature and for time period as before indicated for each microorganism.

Morphological characterisation of microorganisms. Microorganisms of purified colonies were studied for 5 different morphological characters stereomicroscopic feature, colour, margin of colony, surface form, surface texture, elevation (Cruickshank *et al*., 1975). Microorganisms were identified by Gram staining (Murray *et al.,* 1994).

Biochemical tests for microorganisms. Biochemical tests for microorganisms like catalase production, oxidase production, nitrate reduction, triple sugar iron test, and sugar fermentation were also performed. All procedures for biochemical tests were taken from Handbook of Microbiology (Bisen and Verma, 1994).

Economic analysis. Procedures for economic analysis were adopted from CIMMYT (1988). Expenditures incurred on various fertilizing materials and incomes from the respective treatments were calculated on the basis of market rates at the time of experiment. Further, the net return was obtained by finding the difference between total income and total expenditure of each treatment. Finally, the value to cost ratio (VCR) was calculated for each treatment through this formula:

$$\text{Value cost ratio} = \frac{\text{Expenditure on plant nutrients}}{\text{Total income}}$$

Statistical analysis. The data collected for various characteristics were analysed by application of statistical techniques including analysis of variance (ANOVA), and the treatments means were compared by application of Duncan's multiple range test (Sokal and Rohlf, 1997).

RESULTS

Biometric parameters

Vegetative plant growth parameters like plant height, leaf area and biomass yield were measured before harvesting the maize fodder as given in Table 1. Data show that NPK fertilizers gave the highest values for these parameters, which had statistically significant difference with the plants from other treatments. It was followed by BPF+full NP treatment which had statistically non significant difference with full dose of NP fertilizer. The biofertilizers when combined individually with half dose of NP fertilizer could not give plant growth similar to full NP or NPK fertilizer. All these biofertilizers were similar in their effect on plant growth. However, when two biofertilizers were applied together with half NP fertilizer, they could produce plants with height statistically similar to full dose of NP fertilizer. Thus treatments of Biopower+BPF+half NP and Biopower+EM+half NP were not significantly different from full NP. Biopower or EM in combination with half NP fertilizer was statistically superior to only half dose of NP fertilizer. All GFC treatments had lower values than other treatments. Half GFC (SC)+Biopower+EM used in combination had not significantly higher value than other GFC treatments. Full GFC (SC) (500 kg ha^{-1}) gave the lowest fresh fodder yield among all the bio, organic and chemical fertilizer treatments. The plant growth with half GFC (SC)+Biopower+EM was, however, statistically equal to that with half dose of NP fertilizer.

Nutrients uptake

Nitrogen uptake by crop is shown in Table 2; it had statistically different values for various treatments. The highest N uptake was observed in full NPK fertilizer treatment (45.0 kg ha^{-1}) which was followed by BPF+full NP (40.7 kg ha^{-1}) with a significant difference. Full NP fertilizer occupied the third position; however it had statistically non sig-

TABLE 1 - Comparison of organic, bio and chemical fertilizers for growth and yield of maize fodder

Treatments description	Plant height (cm)	Leaf area (cm^2)	Fodder yield (kg ha^{-1})
T_1 Full NP (120-90 kg ha^{-1})	163^{c*}	456^{b*}	8732^{b*}
T_2 Full NPK (120-90-60 kg ha^{-1})	175^{a}	472^{a}	9540^{a}
T_3 Half NP (60-45 kg ha^{-1})	150^{ef}	420^{e}	7532^{d}
T_4 BPF+full NP	167^{b}	461^{b}	9170^{ab}
T_5 Biopower+half NP	155de	441^{d}	8156^{c}
T_6 Biopower+BPF+half NP	158^{cd}	451^{bc}	8528^{bc}
T_7 EM+half NP	155de	428^{e}	8264^{c}
T_8 Biopower+EM+half NP	159^{cd}	445^{cd}	8572^{bc}
T_9 Full GFC (NPK) (250 kg ha^{-1})	143^{g}	395^{f}	7620^{d}
T_{10} Full GFC (SC) (500 kg ha^{-1})	132^{h}	370^{h}	6950^{e}
T_{11} Half GFC (SC)+Biopower	140^{g}	384^{g}	7508^{d}
T_{12} Half GFC (SC)+Biopower+EM	145^{fg}	389^{fg}	8026^{cd}

* Means not sharing a common letter in a column differ significantly at $P \leq 0.05$.

TABLE 2 - Comparison of organic, bio and chemical fertilizers for nutrients uptake by maize fodder

Treatments description	Nutrients uptake (kg ha^{-1})		
	Nitrogen	Phosphorus	Potassium
T_1 Full NP (120-90 kg ha^{-1})	36.6^{c*}	7.29^{b*}	30.9^{d*}
T_2 Full NPK (120-90-60 kg ha^{-1})	45.0^{a}	7.99^{a}	45.2^{a}
T_3 Half NP (60-45 kg ha^{-1})	26.7^{e}	4.42^{e}	28.6^{de}
T_4 BPF+full NP	40.7^{b}	8.28^{a}	40.5^{b}
T_5 Biopower+half NP	30.6^{d}	5.28^{d}	31.5^{cd}
T_6 Biopower+BPF+half NP	33.7^{cd}	6.32^{c}	34.9^{cd}
T_7 EM+half NP	31.8^{d}	5.73^{cd}	32.9^{cd}
T_8 Biopower+EM+half NP	34.2^{cd}	6.15^{c}	35.4^{c}
T_9 Full GFC (NPK, 250 kg ha^{-1})	25.9^{e}	5.21^{d}	30.4^{d}
T_{10} Full GFC (SC, 500 kg ha^{-1})	22.0^{f}	3.61^{f}	24.5^{e}
T_{11} Half GFC (SC)+Biopower	24.8^{ef}	4.11^{ef}	27.4^{de}
T_{12} Half GFC (SC)+Biopower+EM	29.2^{de}	4.81^{de}	31.3^{cd}

* Means not sharing a common letter in a column differ significantly at $P \leq 0.05$

nificant difference with Biopower+EM+half NP and Biopower+BPF+half NP. Generally, non-significant differences were observed among the GFC treatments except half GFC (SC)+Biopower+EM under which the N uptake was 29.2 kg ha^{-1} being superior to other GFC treatments.

The highest values of phosphorus uptake (8.28 and 7.99 kg ha^{-1}) were recorded in BPF+full NP and full NPK treatment, respectively with non significant difference (Table 2). These were followed by full NP treatment showing 7.29 kg ha^{-1} P uptake. The treatments (T_6 and T_8) in which bio-fertilizers were used in combination showed non-significant difference between themselves. They had significantly higher values than all the GFC treatments. Among all GFC treatments, half GFC (SC)+Biopower+EM treatment had higher value of phosphorus uptake than others.

The maximum potassium uptake in maize fodder crop was 45.2 kg ha^{-1}, obtained from full NPK (Table 2). The BPF+full NP treatment showed significantly lower value (40.5 kg ha^{-1}) than full NPK but higher than full NP. Full GFC (NPK) treatment resulted in similar K uptake as with full NP, however they were inferior to Biopower and EM treatments (T5-T8) either used alone or combined. Non-significant differences were observed among half NP and all GFC treatments.

Rhizosphere microflora

Diazotrophic bacteria namely *Azotobacter, Azospirillum, Azoarcus* and *Zoogloea,* and phosphate solubilizing bacteria *Bacillus* and *Pseudomonas* were enumerated from the maize plants rhizosphere at active growth stage viz. 60 days after sowing. Data on the count of *Azotobacter* (Table 3) showed that their population was highest in the treatment of half GFC (SC)+Biopower+EM, which differed not significantly with that of half GFC (SC)+Biopower. The lowest count was observed in NP and NPK fertilizer treatments. The treatments receiving any of the biofertilizer Biopower, BPF or EM singly or in combination with each other caused the establishment of higher populations of *Azotobater* in maize rhizosphere. *Azospirillum* count in the rhizosphere was also maximum with Half GFC (SC)+Biopower+EM, which had no statistical difference with that in Biopower+EM+half NP. These were also having the lowest population in chemical fertilizer treatments. The number of other two diazotrophs *Azoarcus* and *Zoogloea* was also higher in the treatments receiving Biopower alone or in combination especially with EM. The biofertilizers gave higher number of rhizosphere microflora in the treatments where organic fertilizers were also applied. Regarding the phosphate solubilizing bacteria *Bacillus* and *Pseudomonas*, their count was statistically higher with the treatments Biopower+BPF+half NP, and Biopower+EM+half NP, respectively. The application of BPF either alone or in combination with other biofertilizers resulted into higher number of *Bacillus* in the rhizosphere of maize. Contrasting to diazotrophs, the populations of P solubilizers were higher in chemical fertilizer treatments as compared to organic ones.

Economic analysis

The economic analysis on the performance of organic and biofertilizers in comparison with chemical fertilizers for maize fodder production was performed as in Table 4. The per hectare expenditure on various treatments showed the highest value of US$ 121.0 for full NPK (120-90-60 kg ha^{-1}) followed by BPF+full NP (US$ 89.7). The lowest expenses were for half GFC (SC)+Biopower (US$ 37.5), half NP fertilizer (US$ 41.5) and half GFC (SC)+Biopower+EM (US$ 41.7). The maximum income (US$ 318.0) was also from full NPK fertilizer as it yielded the highest fresh fodder biomass, and it was followed by BPF+full NP (US$ 305.7). The lowest income was obtained from full GFC (SC) treatment (US$ 231.7). The net profit was maximum with Biopower+EM+half NP (US$ 235.9) and Biopower+BPF+half NP (US$ 231.9), whereas, full GFC (SC) (500 kg ha^{-1}) gave the lowest net profit (US$ 165.0). The value (total income) to cost (total expenditure) ratio (VCR) was also determined to compare various treatments (Table 4). Data showed that full NPK fertilizer gave the lowest VCR (2.63), which was followed by BPF+full NP (3.41). The highest VCR values were obtained from half GFC (SC)+Biopower (6.67) and half GFC (SC)+Biopower+EM (6.42). All the biofertilizer treatments had intermediate VCR figures showing better economic impact than the chemical fertilizers used at full or half application rate.

TABLE 3 - Comparison of organic, bio and chemical fertilizers for count of N-fixing and P-solubilizing microflora in maize rhizosphere (log CFU g^{-1} dry soil).

Treatments	*Azotobacter*	*Azospirillum*	*Azoarcu*	*Zoogloea*	*Bacillus*	*Pseudomonas*
T1 Full NP (120-90 kg ha^{-1})	5.27^{f}	4.41^{d}	3.44^{de}	4.09^{d}	3.64^{de}	3.58^{d}
T2 Full NPK (120-90-60 kg ha^{-1})	5.18^{f}	4.47^{d}	3.46^{d}	4.13^{d}	3.69^{cd}	3.64^{cd}
T3 Half NP (60-45 kg ha^{-1})	5.38^{ef}	4.51^{d}	3.34^{d}	4.16^{cd}	3.54^{ef}	3.42^{e}
T4 BPF+full NP	5.40^{e}	4.44^{d}	3.55^{cd}	4.14^{cd}	3.87^{ab}	3.69^{c}
T5 Biopower+1/2 NP	5.61^{c}	4.74^{b}	3.69^{b}	4.61^{b}	3.73^{c}	3.57^{d}
T6 Biopower+BPF+1/2 NP	5.66^{bc}	4.76^{b}	3.72^{ab}	4.64^{b}	3.89^{a}	3.79^{ab}
T7 EM+half NP	5.50^{d}	4.66^{c}	3.56^{cd}	4.28^{c}	3.81^{b}	3.76^{b}
T8 Biopower+EM+1/2 NP	5.68^{b}	4.80^{ab}	3.74^{ab}	4.69^{ab}	3.83^{b}	3.83^{a}
T9 Full GFC (NPK, 250 kg ha^{-1})	5.39^{e}	4.50^{d}	3.50^{d}	4.21^{cd}	3.42^{f}	3.33^{e}
T10 Full GFC (SC, 500 kg ha^{-1})	5.51^{d}	4.60^{cd}	3.58^{c}	4.29^{c}	3.55^{e}	3.40^{e}
T11 1/2 GFC (SC)+Biopower	5.69^{ab}	4.79^{b}	3.75^{a}	4.67^{ab}	3.53^{ef}	3.58^{d}
T12 1/2 GFC (SC)+Biopower+EM	5.73^{a}	4.84^{a}	3.77^{a}	4.70^{a}	3.65^{d}	3.64^{cd}

* Means not sharing a common letter in a column differ significantly at $P \leq 0.05$

TABLE 4 - Economic comparison of organic, bio and chemical fertilizers use for maize fodder production

Treatments	Expenditure[1] (US$ ha^{-1})	Fodder Yield (t ha^{-1})	Income[2] (US$ ha^{-1})	Net Return (US$ ha^{-1})	VCR[3]
T1 Full NP (120-90 kg ha^{-1})	83.0	8.73	291.1	208.1	3.51
T2 Full NPK (120-90-60 kg ha^{-1})	121.0	9.54	318.0	197.0	2.63
T3 Half NP (60-45 kg ha^{-1})	41.5	7.53	251.1	209.6	6.05
T4 BPF+full NP	89.7	9.17	305.7	216.0	3.41
T5 Biopower+1/2 NP	45.7	8.16	271.9	226.2	5.95
T6 Biopower+BPF+1/2 NP	52. 3	8.53	284.3	231.9	5.43
T7 EM+half NP	45. 7	8.26	275.5	229.8	6.03
T8 Biopower+EM+1/2 NP	49.8	8.57	285.7	235.9	5.73
T9 Full GFC (NPK, 250 kg ha^{-1})	62.5	7.62	254.0	191.5	4.06
T10 Full GFC (SC, 500 kg ha^{-1})	66. 7	6.95	231.7	165.0	3.48
T11 1/2 GFC (SC)+Biopower	37.5	7.51	250.3	212.8	6.67
T12 1/2 GFC (SC)+Biopower + EM	41. 7	8.03	267.5	225.9	6.42

[1] Expenditure on fertilizer sources: N, P, K (US$ kg^{-1}) = 0.32, 0.50, 0.63, respectively (NFDC, 2004); BPF (US$ 0.67 kg^{-1}); Biopower (US$ 1.67 kg^{-1}); EM (US$ 0.33 L^{-1}; GFC (NPK, US$ 0.25 kg^{-1}); GFC (SC, US$ 0.13 kg^{-1}).
[2] Income of fresh fodder (US$ 33.3 per tonne).
[3] VCR, Value cost ratio.

DISCUSSION

This field study was envisaged with the view to find out some combination of biofertilizers with half dose of chemical fertilizer to have the same effect on crop growth as with the full dose of NPK chemical fertilizers. The results on fodder yield depicted that BPF+full NP treatment performed equally good as did the full dose of NPK fertilizers. Biopower+BPF+half NP and Biopower+EM+half NP treatments gave statistically equal yield as with full dose of NP fertilizers, and better than as with half dose of NP fertilizer. Khaliq *et al.* (2006) reported that integrated use of OM (farm yard manure)+EM with 1/2 mineral NPK yielded similar to the yield obtained from full recommended NPK, indicating that this combination can substitute for 85 kg N ha^{-1}. The BPF contains potassium solubilizing bacteria *Bacillus mucilaginous*, Biopower includes four types of N-fixing bacteria, and EM is having a large number of microbes with major groups as lactic acid bacteria, photosynthetic bacteria, ray fungi and yeast (Higa and Wididana, 1991). These microorganisms are well known to have beneficial effect on plant growth and their nutrient accumulation. Panwar *et al*. (2000) observed that biofertilizers increased leaf area, chlorophyll concentration and total biomass production in wheat. Recently, the concept of microbial consortium in the production and use of biofertilizers is emerging strongly. This is due to the reason that some beneficial microorganism having similar ecological requirements flourish in an associative manner (Vessey, 2003). Ponnuswamy *et al.* (2002) reported that a combination of phosphobacteria+*Azospirillum* had positive effect on yield and yield characters compared to no biofertilizer application and with phosphorus solubilizing bacteria (PSB) or *Azospirillum* alone. These findings coincide with results of Nanda *et al.* (1995) who observed that green fodder yield and benefit-cost ratio (VCR) were highest with a combination of 75 kg N ha^{-1} and seed inoculation with *Azospirillum* (22.0 t ha^{-1} yield and 1.37 VCR) and lowest in the control (7.6 t ha^{-1} yield and 0.07 VCR). The results of present study are also supported by Rout *et al*. (2001) who found that *Azotobacter, Azospirillum* and their combinations gave more yield than untreated maize. They concluded that biofertilizers in combination with inorganic nitrogen fertilizers can substitute up to 20 % nitrogenous fertilizers and can increase maize yield. Similarly, Mokhova *et al.* (2000) reported that application of Rhizobacterin (inoculum containing mixed culture of N-fixing and P-solubilizing bacteria) resulted in yield increases and required lower nitrogen fertilizer rates.

The uptake or accumulation of the macronutrients like N, P and K is the direct reflection of the biomass production. The result of this experiment revealed that full dose of NPK caused the maximum uptake of N, P and K nutrients by maize fodder, and it was followed by BPF+full NP, even better P uptake than from the chemical fertilizers. Biofertilizer treatments especially those with Biopower improved the NPK uptake over half dose of NP fertilizer. This was due to the fact that Biopower contained four different species of N-fixing bacteria, which resulted into increased availability of nitrogen to the crop. Wu *et al.* (2005) indicated that half the amount of biofertilizer application had similar effects when compared with organic fertilizer or chemical fertilizer treatments. Microbial inoculum not only increased the nutritional assimilation of plants (total N, P and K), but also improved soil properties, such as organic matter content and total N in soil. Elshanshorey (1995) also reported that biofertilizers increased nutrient concentration and uptake by cereal crops, which lead towards luxurious growth and better crop development. Combination of N fertilizer with EM also increased the concentrations of NPK in plants (Khaliq *et al.,* 2006). Phosphorus uptake was highest under BPF+full NP treatment, even better than full dose of NP or NPK treatments. In fact BPF has *Bacillus mucilaginous* bacteria which have solubilizing effect on fixed phosphorus in the soil. Son *et al*. (2006) enlisted a number of microorganisms which have the ability to solubilize phosphorus in the soil and make it available to plants. The organic fertilizers viz. GFC either with or without NPK and microbial inoculants gave very little P uptake, it was due to the reason that the amount of GFC was so little that it could not provide phosphorus as much needed by the crop. Potassium uptake was also highest under full NPK treatment having statistical difference even with BPF+full NP which gave significantly better K uptake than with full NP fertilizers. As NP alone treatment did not contain K in it, so the uptake was lower. The BPF as apparent from its name i.e. Biological Potassium Fertilizer contained bacteria *Bacillus mucilaginous* which had a strong ability to make fixed potassium available from the soil minerals to crops. The results are supported by Duraisami and Mani (2000) who stated that the inorganic, organic and biological nitrogen sources also enhance the uptake of P and K under maize. Khaliq *et al*. (2006) observed that combination of both N sources with EM increased the NPK concentrations in cotton crop.

The populations of diazotrophs as well as P solubilizers were affected significantly with the application of biofertilizers, and they were also variable with the types and combinations of the applied biofertilizers. Generally, the number of diazotrophic bacteria was enhanced more with Biopower, as it contained all the four enumerated bacteria which were colonized in the rhizosphere due to their inoculation on the seed. The results exactly coincide with findings of Khokar (2004) who conducted research on interaction of indigenous *Azospirillum* with maize and observed that the highest root colonization was under inoculum treatments. The application of EM and BPF increased the number of diazotrophs further, although their inoculum did not contain any of the enumerated bacteria. This might be due to the positive interactive effect of the microbes in these biofertilizers with N-fixing bacteria. These results were in conformity with Mehnaz *et al.* (1998) who conducted research on detection of inoculated plant growth promoting rhizobacteria in rice and isolated strains belonging to *Azospirillum, Azoarcus, Pseudomonas* and *Zoogloea.* They found that the populations of these bacteria as well as that of indigenous ones were more under inoculum treatments as compared to uninoculated. The organic fertilizers were better than chemical fertilizer in terms of increasing the number of N-fixing bacteria in the rhizosphere. These results indicate that microbial population of soil can be increased by applying organic matter and their effectiveness can be increased by inoculation with biofertilizer. In this way availability of nutrients can be increased to plant and maximum yield can be achieved (Elshanshorey, 1995). In a previous study by Ali *et al.* (1998) on the bacterial counts under farm yard manure and chemical fertilizers treatments, they found that diazotrophs, in presence or absence of NP fertilizer did not respond significantly to inoculation in terms of N content. The count of P solubilizers especially that of *Bacillus* was higher under BPF treatment, which was due to the reason that BPF contained *Bacillus mucilaginous* already in the inoculum. Application of EM also had positive effect to increase the number of phosphorus solubilizing bacteria in the maize rhizosphere. This was probably due to the reason that the yeast and photosynthetic bacteria contained in EM released some biochemical compounds which enhanced the growth and population of N-fixing as well as P-solubilizing bacteria in rhizosphere. Similarly, Ren *et al.* (2006) reported that a bacterium strain B-916 of *Bacillus subtilis,* caused growth promotion in roots and leaves of two rice varieties in addition to inducing disease resistance.

The economic analysis of data revealed highest expenses and total income with NPK fertilizer due to the reason that it produced the highest yield of fodder. However, chemical fertilizer treatment couldn’t give a better net return and VCR, rather it was among the lowest. In this regard, the organic and biofertilizers yielded better economic returns due to their less cost as compared with chemical fertilizers. Biopower+BPF or EM+half NP were proved to be the best treatments in terms of economics. This was due to low cost of these biofertilizers and their efficiency on plant growth. Wu *et al.* (2005) indicated that half the amount of biofertilizer application had similar effects when compared with organic fertilizer or chemical fertilizer treatments.

CONCLUSIONS

The use of BPF+full NP gave fodder maize growth, yield and nutrients uptake equal to that with full dose of NPK fertilizers. However, Biopower and EM along with half dose of NP also showed results similar to that with full dose of NPK. Therefore, the study concluded that the beneficial microorganisms / biofertilizers applied in combination were a better choice for farmers to reduce the use of chemical fertilizers for sustainable crop production. The combined use of Biopower+EM+half NP, was also found to give more economic return. Further, the organic fertilizers or composts applied in low quantity are insufficient to meet the nutrients requirement of the crop even if with biofertilizers, so they need to be applied in bulk (tonnes) quantities. The combined use of bio and organic fertilizers along with chemical fertilizers is more economical in terms of crop yields per unit area, and it is also a sustainable crop production technology.

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