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EFFECTS OF BIOFERTILIZERS COMBINED WITH DIFFERENT SOIL AMENDMENTS ON POTTED RICE PLANTS

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ABSTRACT

This pot study investigated the effect of the combined application of two commercial biofertilizers viz. Biopower and EM (Effective Microorganisms) on rice (*Oryza sativa* L.) growth and yield in soils amended with farmyard manure, green manure, and NPK fertilizers. Biopower is a product of the Nuclear Institute for Biotechnology and Genetic Engineering (NIBGE), Pakistan, which contains species of associative and endophytic diazotrophs. EM (effective microorganisms), a product developed by Japanese scientists, consists of co-existing beneficial microorganisms, mainly species of photosynthetic and lactic acid bacteria, as well as yeast. Applying Biopower adversely affected plant growth and yield in NPK fertilizer amendment. Conversely, this biofertilizer markedly enhanced plant growth and yield in green manure amended soil while its effect was not significant in farmyard manure amendment. In green manure amendment, applying EM enhanced grain yield by 46%. Co-inoculation of Biopower and EM evidently improved root and shoot growth in farmyard manure amended soil. This study concludes that the two biofertilizers clearly enhanced shoot biomass and grain yield in green manure amended soils.

Key words: Associated N_2-fixers, Biopower, effective microorganisms, rice, soil amendments.

INTRODUCTION

Rice (*Oryza sativa* L.) is probably the most important cereal in the world and serves as food for about 50% of the world's population (Ladha *et al., 1997*). This crop requires about 10 million tons of N fertilizers every year worldwide (IRRI, 1996). Rice is usually grown in N-deficient soils, and this element must be supplied to the field by commercially available N fertilizers. However, a substantial amount of urea-N or NO_3-N applied as fertilizers is lost through different mechanisms, thus causing environmental pollution problems. Use of biological N_2-fixation technology can decrease N fertilizer application and reduce environmental risks (Raimam *et al.,* 2007). This process can contribute as much as 75 kg N ha^{-1} per crop cycle with means of 8 to 30 kg N ha^{-1} (Irissarri and Reinhold-Hurek, 2001). These N-fixing bacteria may be free-living or naturally associated to rice plants. Recent studies have shown that several bacteria may be isolated from sterilized surface roots of flooded rice plants, suggesting endophytic colonization (Raimam *et al.,* 2007). The most likely candidates for biological N fixation in rice are species of *Alcaligenes*, *Azospirillum*, *Bacillus*, *Enterobacter*, *Herbaspirillum*, *Klebsiella*, *Pseudomonas*, and *Rhizobium* (Malik *et al.,* 1997; James *et al.,* 1999; Yanni *et al.,* 1997). From the soil and plant materials collected from different areas of Pakistan, scientists of the Nuclear Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan have isolated a number of bacterial strains belonging to the *Azospirillum*, *Azotobacter*, *Pseudomonas*, and *Zoogloes genera*. After laboratory testing and studying field performance, efficient bacterial strains for biofertilizer production were finally selected.A suitable carrier material was selected after trying a number of local materials to ensure maximum survival of the inoculated bacteria during storage and transportation. They named this biofertilizer Biopower.

EM (effective microorganisms) technology of nature farming was introduced by Higa (1991). EM culture consists of co-existing beneficial microorganisms, mainly species of photosynthetic bacteria (*Rhodopseudomonas plastris* and *Rhodobacter sphacrodes*), lactobacilli

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(*Lactobacillus plantarum*, *L. casei*, and *Streptococcus lactis*), yeast (*Saccharomyces* spp.), and actinomycetes (*Streptomyces* spp.), which improve crop growth and yield by increasing photosynthesis, producing bioactive substances, such as hormones and enzymes, controlling soil diseases, and accelerating decomposition of lignin materials in the soil (Higa, 2000; Hussain *et al.,* 2002). This technology of nature farming was introduced in Pakistan in 1990 by the Nature Farming Research Centre of the University of Agriculture, Faisalabad. Numerous field and greenhouse trials indicate the benefits of EM as a biofertilizer in crop production, as a probiotic in poultry and livestock rations, and as a starter to improve composting and recycling of municipal/industrial wastes and effluents (Hussain *et al*., 1999). There are controversial reports about the effect of applying EM on crop growth and yield. Many researchers have reported an increase in crop growth and yield by applying EM (Daly and Stewart, 1999; Yan and Xu, 2002; Javaid, 2006; 2009; Khaliq *et al.,* 2006; Javaid and Mahmood, 2010). However, other researchers have revealed that the effect of EM on crop growth and yield was not usually evident or even negative, especially in the first test crop (Bajwa *et al.,* 1999; Daiss *et al.,* 2008; Javaid *et al.,* 2008, Javaid and Shah, 2010).

Earlier researchers conducted studies by using either effective microorganisms or associative N_2-fixing organisms. However, there are no studies on the effect of combined inoculation of these two biofertilizers on rice growth and yield. Therefore, this pot study investigated the effect of the combined application of effective microorganisms and Biopower on rice growth and yield in soil amended either with NPK fertilizers or two types of organic manures viz. farmyard and green.

MATERIALS AND METHODS

Soil characteristics

Loamy textured soil with organic matter 0.9%, pH 7.8, 0.045% N, 14 mg kg^{-1} available P, and 210 mg kg^{-1} available K was employed in the experiment. Micronutrients Fe, Cu, and Zn were 9.53, 1.71, and 4.42 mg kg^{-1} of soil, respectively. The experiment was conducted in the Botanical Garden of the University of the Punjab, Lahore (31.57° N, 74.31° E), Pakistan.

Soil amendments

Earthen pots, 20-cm diameter and 30-cm depth, were filled with 5 kg soil. Soil was amended either with farmyard manure at 4 g 100 g^{-1}, *Trifolium alexandrianum* green manure at 3 g 100 g^{-1} (on dry weight basis), or NPK fertilizers. A basal dose of 40 mg kg^{-1} N as urea (half of recommended dose), 30 mg kg^{-1} P_2O_5 as triple superphosphate, and 20 mg kg^{-1} K_2O as potassium sulfate was supplied to NPK treatment pot soil. NPK fertilizers were mixed 3 d prior to sowing. All pots were irrigated with tap water and left for 20 d for organic material decomposition.

Treatments and experimental design

There were four treatments, with three replicates, for each of the three soil amendment systems. These were; i) control, ii) effective microorganisms, iii) Biopower, and iv) Biopower + effective microorganisms. The experimental design was completely randomized. Pots were arranged on a bench in a wire-netting greenhouse under natural environmental conditions.

Effective microorganism application

The effective microorganism (EM) culture, commercially known as EM Bioaab, was obtained from the Nature Farming Research and Development Foundation, Faisalabad, Pakistan. EM contained high populations of lactic acid bacteria at 1×10^{11} cfu mL^{-1}, photosynthetic bacteria at 1×10^{6} cfu mL^{-1}, and 1×10^{3} cfu mL^{-1} yeast suspension (Higa, 2000). Stock culture was diluted by adding tap water to prepare a 0.2% solution. The fresh solution was used immediately. The respective treatment pots with effective microorganisms applied in all three soil amendment systems were irrigated with a 0.2% diluted EM solution 15 d prior to sowing. Each pot received 1 L of diluted EM solution. These pots were further supplemented with 1 L of 0.2% EM solution every 2 wk throughout the experimental period (Yadav, 2002).

Biopower application

Commercial Biopower was obtained from the Nuclear Institute for Biotechnology and Genetic Engineering, Faisalabad, Pakistan. Biopower was mixed in 100 g L^{-1} water. One-month-old rice plants var. Super Basmati were immersed in the Biopower solution for 1 h and transplanted three plants per pot. All pots were flooded with tap water. Flooded conditions were maintained throughout the experimental period, except during the last month.

Harvesting

Plants were harvested 90, 120, and 150 d after sowing. Shoot length was measured and number of tillers was counted for each plant. Root and shoot materials were dried at 60 ºC until constant weight and then weighed. Grain yield per plant and 100-grain weight were also recorded at final harvest.

Statistical analysis

There were three replicates for each treatment, and mean data per plant was calculated for various studied parameters. Data of various root and shoot growth, as well as yield parameters were subjected to ANOVA. This was followed by Duncan's Multiple Range Test with the SPSS and CoStat software to delineate mean differences (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Effect of biofertilizers and soil amendments on plant vegetative growth

ANOVA shows that the effect of soil amendments (A) was significant for all root and shoot growth parameters at the three growth stages (Tables 1 and 3). In general, plant growth was better in farmyard manure than in NPK fertilizers and green manure amendments (Table 2). Except in some cases, the general effect of EM and Biopower (B) was not significant for various vegetative growth parameters at different growth stages. The interactive effect of A × B and A × EM was significant for root biomass at the 90 d growth stage. Similarly, the effect of A × EM was significant for shoot length at the 120 and 150 d growth stages. The tripartite interactive effect of A × B × EM was significant for shoot length at all three growth stages, and for root biomass at 120 and 150 d (Tables 1 and 3).

Effect of the two biofertilizers on shoot length was not significant in all three soil amendment systems at the 90 d growth stage. At 120 d, combined inoculation of the two biofertilizers markedly enhanced shoot length in NPK and green manure amended soils. Applying Biopower at the final growth stage significantly enhanced shoot length in green manure and farmyard manure amended soils. EM applied either alone or combined with Biopower did not result in a significant increase in shoot length (Table 2).

Number of tillers in different soil amendment systems varied at the first two growth stages with the effect of single as well as combined inoculation of the two biofertilizers. In general, applying biofertilizer either reduced tillering or exhibited no effect on this parameter. However, at the final growth stage, the number of tillers significantly increased with EM application, either alone or combined with Biopower, in the farmyard manure amendment (Table 2).

At different growth stages, shoot biomass either declined or remained unaffected due to separate or combined inoculation of the two biofertilizers in NPK fertilizer amended soil. In contrast, in the green manure amendment, both biofertilizers generally increased shoot biomass at all three growth stages. The effect of both biofertilizers was not appreciable at the 90 and 120 d growth stages in the farmyard manure amendment. However, at the 150 d growth stage, a marked increase in shoot biomass was recorded by both biofertilizers. The effect of combined inoculation was more evident than when they were used alone (Table 2).

Variable response of root biomass to two applied biofertilizers was recorded in different soil amendment systems. There was no definite pattern of root biomass production at different growth stages in response to either of the two biofertilizer treatments in NPK fertilizers. Both biofertilizers generally enhanced root biomass at different growth stages in the green manure amendment. In the farmyard manure amendment, after an initial decline at

Table 1. ANOVA for the effect of soil amendments, biopower, and EM application on various rice vegetative growth parameters after 90 and 120 d growth stages.

		90 d after sowing				120 d after sowing			
Trait	df	Shoot length	N° of tillers per plant	Shoot biomass	Root biomass	Shoot length	N° of tillers per plant	Shoot biomass	Root biomass
Treatments	11	48^{ns}	104***	29***	22***	127**	70***	466***	122***
Amendments (A)	2	134**	517***	145***	91***	196*	356***	178***	523***
Biopower (B)	1	0.22^{ns}	17ns	1.16^{ns}	0.58^{ns}	51^{ns}	1.78^{ns}	0.41^{ns}	2.26^{ns}
EM	1	13^{ns}	47**	0.05^{ns}	1.14^{ns}	0.03^{ns}	0.00^{ns}	0.03^{ns}	0.04^{ns}
A × B	2	4.4^{ns}	5.9^{ns}	4.29^{ns}	20***	61^{ns}	2.53^{ns}	1.79^{ns}	9.94^{ns}
A × EM	2	12^{ns}	8.7^{ns}	8.49^{ns}	6.2*	179*	0.25^{ns}	2.67^{ns}	13.2^{ns}
B × EM	1	28	0.25^{ns}	0.12^{ns}	0.02^{ns}	72^{ns}	16^{ns}	1.03^{ns}	88**
A × B × EM	2	93*	7.6^{ns}	2.8^{ns}	3.4^{ns}	199*	17^{ns}	0.89^{ns}	81***
Error	24	27	6.3	3.24	1.5	45	7.7	178	7.8
Total	36								

*, **, ***: significant at P≤ 0.05, 0.01, and 0.001, respectively.
ns: non significant.

Table 2. Effect of soil amendments, biopower, and EM application on various rice vegetative growth parameters after 90, 120, and 150 d growth stages.

	90 d after sowing				120 d after sowing				150 d after sowing			
Treatments	SL	TPP	SB	RB	SL	TPP	SB	RB	SL	TPP	SB	RB
	cm	g			cm	g			cm	g		
NPK fertilizers												
Control	57.0a-c	9cd	3.90de	2.11d	91a-c	9c	19.5bc	4.4d	88b-d	11c	17.4cd	6.7c
BP	50.2c	10c	2.87de	4.96bc	84bc	9c	16.0c	5.3d	82cd	8c	9.8d	5.7c
EM	51.5bc	7cd	1.45e	1.53d	82c	10c	19.0bc	3.4d	79d	10c	15.2cd	4.8c
BP+EM	57.8a-c	5d	2.42de	1.78d	100a	9c	22.2bc	3.9d	84cd	10c	14.9cd	11.4c
Green manure												
Control	57ac	10c	3.0de	1.81d	90a-c	10c	18.7bc	4.7d	88bd	11c	52.5bc	7.5c
BP	55ac	9cd	4.5de	2.33d	96ab	10c	21.5bc	4.6d	100a	12c	60.3b	17.5c
EM	57ac	9cd	5.7bd	2.33d	96ab	7c	19.2bc	6.7cd	94ac	11c	59.3bc	18.1c
BP+EM	61ab	9cd	5.5cd	3.25cd	100a	10c	24.3b	7.6cd	98ab	9c	50.8bd	12.0c
Farmyard manure												
Control	60ac	23a	10.1a	9.3a	102a	20ab	47a	23a	89b-d	20b	125a	48b
BP	63a	19ab	8.9ab	5.3bc	102a	18ab	46a	11bc	102a	20b	141a	45b
EM	63a	19ab	10.1a	8.5a	96ab	16b	44ab	13b	92a-c	24a	156a	57ab
BP+EM	58a-c	17b	8.2a-c	6.1b	90a-c	21a	42a-c	19a	93a-c	25a	159a	75a

Values with different letters in a column are significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).
SL: Shoot length; TPP: Tillers per plant; SB: Shoot biomass; RB: Root biomass; BP: Biopower; BP+EM: Biopower+EM; EM: effective microorganisms.

Table 3. ANOVA for the effect of soil amendments, biopower, and EM application on various rice vegetative and reproductive growth parameters at maturity (150 d after sowing).

		Mean squares					
Trait	df	Shoot length	N° of tillers per plant	Shoot biomass	Root biomass	Grain yield	100-grain weight
Treatments	11	957^{***}	92^{***}	5596^{***}	836^{***}	105^{***}	0.050^{***}
Amendments (A)	2	734^{***}	1426^{***}	$28\,921^{***}$	5137^{***}	432^{***}	0.158^{***}
Biopower (B)	1	174^{*}	5.8^{ns}	16^{ns}	29^{ns}	12^{ns}	0.024^{ns}
EM	1	0.653	2.7^{ns}	118^{ns}	170*	13^{ns}	0.002^{ns}
A × B	2	40^{ns}	0.93^{ns}	32^{ns}	3.3^{ns}	20^{ns}	0.035^{*}
A × EM	2	131*	2.8^{ns}	152^{ns}	91^{ns}	55^{**}	0.052^{**}
B × EM	1	27^{ns}	3.3^{ns}	20^{ns}	87^{ns}	5^{ns}	0.004^{ns}
A × B × EM	2	381^{***}	14.8^{ns}	83^{ns}	183^{**}	57^{**}	0.002^{ns}
Error	24	39	5.9	149	39	11	0.011
Total	36						

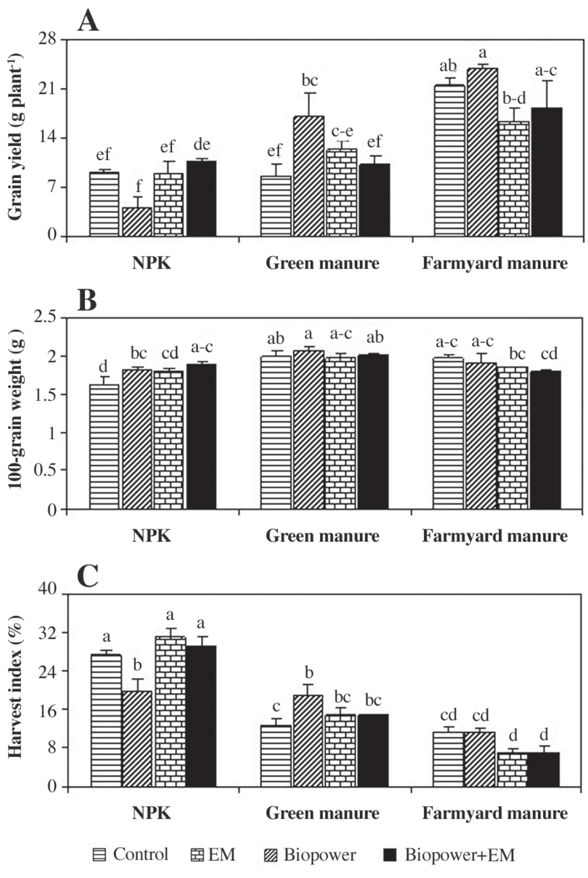
*, **, *** significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.
ns: non significant.

the 90 and 120 d growth stages due to both biofertilizers, root biomass was enhanced by EM and EM + Biopower applied at the 150 d growth stage. Effect of combined inoculation of the two biofertilizers was much more evident than EM applied alone (Table 2).

Effect of biofertilizers and soil amendments on plant reproductive growth

ANOVA shows that the effect of soil amendment was significant for grain yield and 100-grain weight. In contrast, effects of EM and Biopower were not significant for both these reproductive growth parameters. Effect of A × B was significant for 100-grain weight while A × EM was significant for both grain yield and 100-grain weight. Tripartite interaction of A × B × EM was significant for grain yield (Table 3).

Grain yield showed a variable response to Biopower and EM in different soil amendments. Applying Biopower in the NPK fertilizer amendment resulted in a 55% reduction in grain yield. Conversely, in the green manure amendment, a significant 99% increase in grain yield was recorded by applying Biopower. The effect of applying Biopower in the farmyard manure amendment was not observable (Figure 1A). EM application enhanced grain yield by 46% in the green manure amendment. In contrast, there was a 24% reduction in grain yield by applying EM in farmyard manure. The effect of combining EM and Biopower was not significant in none of the three soil amendment systems (Fig. 1A). Earlier studies depicted variable effects of EM application on crop growth and yield; some researchers have shown positive effects of EM (Javaid, 2006; Khaliq *et al.,* 2006; Javaid and Bajwa, 2010) while others reported negative or no effects (Bajwa *et al.,* 1999; Formowitz *et al.,* 2007; Daiss *et al.,* 2008). According to Kinjo *et al.* (2000), the lack of consistency in experimental results when applying EM may be due to variable cultural conditions employed in previous studies. However, this study reveals that the effect of applying EM on crop growth and yield is associated with the type of soil amendment used.



Values with different letters at the top of the bars are significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

Figure 1. Effect of Biopower and EM application on rice grain yield, 100-grain weight, and harvest index in different soil amendment systems. Vertical bars show standard error of means of three replicates.

Inoculation of EM and Biopower, either alone or combined, enhanced 100-grain weight in the NPK fertilizer amendment. The effect of Biopower and combined inoculation was significant. In contrast, applying biofertilizer to farmyard manure reduced 100-grain weight. In green manure amended soil, the effect of either biofertilizer was not significant (Figure 1B). The effect of the two biofertilizers on the harvest index in different soil amendment systems was generally similar to effect on grain yield (Figure 1C).

CONCLUSIONS

This study concludes that the beneficial effects of EM and Biopower can best be exploited by using them combined with a suitable soil amendment. Both of these biofertilizers markedly enhanced rice shoot biomass and grain yield in the green manure amendment.

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RESUMEN

Efecto de biofertilizantes combinado con enmiendas del suelo sobre plantas de arroz en macetas. El presente estudio en macetas fue realizado para investigar el efecto de la aplicación combinada de dos biofertilizantes comerciales, Biopower y EM (Effective Microorganisms), en el crecimiento y producción de arroz (*Oryza sativa* L.) en suelos enmendados con estiércol de granja, abono verde y fertilizantes NPK. Biopower es un producto del Nuclear Institute for Biotechnology and Genetic Engineering (NIBGE), Pakistán, que contiene especies de diazótrofos asociativos y endofíticos. Effective Microorganisms es un producto desarrollado por científicos japoneses que consiste en microorganismos benéficos co-existentes, principalmente especies de bacterias fotosintéticas y ácido lácticas, y levadura. La aplicación de Biopower afectó adversamente el crecimiento y producción vegetal en enmienda de fertilizante NPK. Inversamente, este biofertilizante mejoró marcadamente el crecimiento y producción vegetal en suelo enmendado con abono verde mientras en enmienda con estiércol de granja su efecto fue insignificante. En enmienda con estiércol verde, la aplicación de EM mejoró el rendimiento de grano en 46%. Co-inoculación de Biopower y EM mejoró marcadamente el crecimiento radical y de brotes en suelo enmendado con estiércol de granja. El presente estudio concluye que los dos biofertilizantes mejoraron marcadamente la biomasa de brotes y rendimiento de grano en suelos enmendados con abono verde.

Palabras clave: fijadores de N_2 asociados, Biopower, microorganismos efectivos, arroz, enmiendas de suelo.

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