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EFFECT OF SOIL AMENDMENTS, WEEDS AND BIOPOWER APPLICATION ON MYCORRHIZAL COLONIZATION IN RICE

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ABSTRACT

A field study was undertaken to investigate the effect of weeds, soil amendments and Biopower (a commercial biofertilizer) on vesicular arbuscular mycorrhizal (VAM) colonization in rice (*Oryza sativa* L.) var. Basmati Super. i)- Field soil was amended with recommended doses of NPK fertilizers, ii)- half N and recommended doses of P and K fertilizers, and iii)- farmyard manure. Four common rice weeds namely *Cyperus rotundus* L., *Echinochloa colonum* (L.) Link, *Paspalum paspaloides* (Michx.) Scribn. and *Marsilea minuta* L. were planted as 1:1 weed rice plant ratios in all the three soil amendments. All the treatments were with or without Biopower application. Mycorrhizal colonization was studied after 50, 80 and 110 days of rice transplantation corresponding to various growth stages viz. vegetative, flowering/panicle and maturity, respectively. The highest mycorrhizal colonization in rice was recorded at panicle stage i.e. 80 days after transplantation. Mycorrhizal colonization in rice varied with weed species, soil amendment and growth stage of rice. Generally, weeds stimulated mycelial, arbuscular and vesicular colonization in rice roots. The highest stimulatory effect on mycorrhizal colonization was due to mixed weeds in all the three soil amendments at various growth stages of the crop. Biopower application significantly enhanced mycorrhizal colonization in weed free treatment or rice plants co-cultivated with *P. paspaloides* and *M. minuta* in one or the other soil amendment.

Keywords: Biopower, mycorrhiza, rice, soil amendments, weeds.

INTRODUCTION

Rice is the second largest staple food crop in Pakistan, cultivated all over the fertile lands in Sindh and Punjab (PARC, 2006; Shafique and Ashraf, 2007). Pakistan is major exporter ranked 6th in world rice production (Abro *et al.*, 2013), while Basmati (high quality aromatic rice) and IRRI (coarse rice) are two well-known extensively cultivated groups (Fatima *et al.*, 2007). Amongst number of rice production constrains in the country, infestation of weed is questionable matter responsible for serious yield reduction annually (Rabbani *et al.*, 2011). However, losses caused by weeds vary with location, weed infestation duration and weed flora predominance (Hakim *et al.*, 2013). A number of weed species including *Cyperus rotundus* L., *C. difformis* L., *Echinochloa colonum* (L.) Link, *Paspalum paspaloides* (Michx.) Scribn. and *Marsilea minuta* L. have been recorded from different rice growing areas in Pakistan (Rabbani and Bajwa,

2001; Mann *et al.*, 2007; Rabbani *et al.*, 2011).

The negative impact of weeds on plant productivity can be accompanied by deleterious consequences on natural beneficial soil microbial flora. In this connection, vesicular arbuscular mycorrhizal (VAM) fungi are important aspect of natural ecosystem that are in fact a bridge like association between root of vascular plant and fungi for the bidirectional transport of nutrients (Javaid, 2009; Bucher *et al.*, 2014). VAM fungi are well-known as profitable agent for growth and yield of majority of the plants including rice thereby enhance disease resistance, drought tolerance, nutrients dynamics and ecology of the host plant (Angelard *et al.*, 2010; Colard *et al.*, 2011; Nadeem *et al.*, 2014). Interestingly, differential response of VAM association with different weed species has been documented including mutualistic, negative or positive (Vatovec *et al.*, 2005; Jordan and Huerd, 2008; Rinaudo *et al.*, 2010). Therefore, VAM fungal-weed interaction may reduce crop yield by promoting weed growth, may alter relative abundance of mycotrophic weeds species thereby increase activities of other soil microbial activities with increase in crop yield (Bilalis and Karamanos,

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2010). So far, there is controversy about the occurrence of mycorrhizal association with rice roots. It was found that rice plants readily form mycorrhizal associations under upland conditions, but under submerged conditions colonization is rare due to the anoxic environment (Ilag *et al.*, 1987). Purakayastha and Chhonkar (2001) documented survival of VAM fungi under submerged conditions. Hajibol *et al.* (2009) investigated that rice plant uptake more amount of insoluble P when inoculated with either *Glomus mosseae* or *G. intraradices* under either flooded or non-flooded conditions. Angelard *et al.* (2010) speculated that specific VAM fungal genotypes could increase the biomass of rice up to five times compared with other isolates. Vallino *et al.* (2014) stated that rice flooding negatively affects root branching and arbuscular mycorrhizal colonization, but not fungal viability or its functionality.

Biopower is a commercial biofertilizer comprised of beneficial microorganisms including live or latent cells of efficient strains of nitrogen fixing, phosphate solubilizing or cellulolytic microorganisms. This product was prepared by scientists of NIBGE Faisalabad, Pakistan. These are extensively used for acceleration of microbial processes and availability of nutrients to plants (Mahdi *et al.*, 2010). Earlier studies have shown variable effects of soil beneficial microorganisms on development of VAM fungi and subsequent effects on crop growth and yield. Most of the studies showed that generally mycorrhizal colonization is favoured by soil beneficial microorganisms (Barea *et al.*, 2002; Hildebrandt *et al.*, 2006; Ruíz-Sánchez *et al.*, 2011; Saxena *et al.*, 2013). However, some studies also reported otherwise (Hetrick *et al.*, 1988; Miransari, 2011). The present study was, therefore, carried out to investigate the effect of weeds and Biopower application on development of VAM fungi in rice.

MATERIALS AND METHODS

Field experiment: To study the effect of soil amendments, Biopower and weeds on arbuscular mycorrhizal colonization in wheat, a field experiment was carried out in split-split plot design with soil amendments as main plots, Biopower as sub-plots and weed species as sub-sub-plots. Each sub-sub plot was measured 1.5 m × 1.5 m. Three soil amendment levels included i)- Recommended dose of NPK (N 120, P₂O₅ 75, K₂O 60 kg ha⁻¹), ii)- half dose of nitrogen along with recommended dose of P and K, and iii)- farmyard manure (FYM) at 15 tons ha⁻¹. One month old rice nursery was transplanted in the field plots with inter and intra row spacing of 20 cm. For Biopower inoculation, rice seedlings

were dipped in Biopower suspension (375 g of Biopower in 4 liters of water) for 30 minutes before transplantation. Seedlings (15 days old) of weeds namely *E. colonum*, *C. rotundus*, *P. paspaloides* and *M. minuta* were transplanted (1:1 ratio of weed and rice) in respective plots 15 days after rice transplantation. In each soil amendment, weed free treatment served as control. Each treatment was replicated thrice. Six rice plants were carefully uprooted from each replicate plot after 50, 80 and 110 days of rice transplantation.

Estimation of mycorrhizal colonization: Fresh rice roots from each treatment were washed thoroughly under tap water and cut into 1-cm pieces. These root pieces were autoclaved for five minutes in 10% KOH solution at 121 °C. Thereafter, roots were washed with distilled water. For staining, roots were autoclaved in trypan blue stain following Phillips and Hayman (1970). Ten 1-cm long root segments of each replicate from all the treatments were placed on glass slide, a cover slip was placed over them and pressed gently. Slides were observed under the microscope for the estimation of mycorrhizal status. Root segments were examined under microscope for assessment of vesicular, arbuscular and mycelial infection. Mycorrhizal colonization was recorded in terms of percentage of arbuscular, vesicular and mycelial infection on the bases of presence or absence of these structures in the root pieces. All the data were statistically analyzed by analysis of variance followed by Duncan's Multiple Range Test at 5% level of significance (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Effect of weeds on mycorrhizal colonization in rice:

In general, mycelial colonization was the highest after 80 days of rice transplantation in all the three soil amendment systems. In recommended dose of N fertilizer, mycelial colonization varied from 20-43%, 40-63% and 13-23% after 50, 80 and 110 days of transplantation, respectively. Likewise, there was 17-43%, 37-60% and 10-20% mycelial colonization in half dose of N fertilizer, and 17-27%, 27-56% and 17-43% mycelial colonization in farmyard manure amendment after 50, 80 and 110 days of rice transplantation, respectively (Table 1-3). These results clearly indicate that mycorrhizal colonization in rice continues till panicle stage and decline at ripening. Although the effect of weeds on mycelial colonization was insignificant statistically, however, generally

colonization was enhanced to variable extents due to weeds infestation. Stimulatory effect of weeds on mycelial colonization varied with weed species, soil amendments and growth stage of the host plant. In general, stimulatory effect of mixed weeds on mycelial colonization was more pronounced than the effect of individual weeds. In farmyard manure amendment, there was 59%, 96% and 153% increase in mycelial colonization in rice roots due to mixed weeds over weed free control after 50, 80 and 110 days of transplantation, respectively. Similarly, there was 59% and 22% increase in mycelial colonization due to mixed weeds in ½ N dose and 59% and 35% increase in recommended dose of N fertilizer over weed free control after 50 and 80 days of transplantation, respectively (Table 1-3). Similar to that of mycelial colonization, arbuscular and vesicular colonizations were also markedly enhanced due to mixed weeds. There was 153% and 39% increase in arbuscular

colonization in ½ N treatment, and 130% and 25% increase in recommended dose of N after 50 and 80 days of rice transplantation, respectively. In case of farmyard manure, mixed weeds increased arbuscular colonization in rice at all the three growth stages. In this soil amendment system, there was 58%, 96% and 300% increase in arbuscular colonization after 50, 80 and 120 days of rice transplantation (Table 1-3). Vesicular colonization was low 50 days after rice transplantation. The highest vesicular colonization was recorded 80 days after transplantation. At this growth stage, there was 117% and 185% increase in vesicular colonization due to mixed weeds in recommended N dose and farmyard manure amendments, respectively (Table 1-3). Earlier studies have shown variable effects of co-cultivated on mycorrhizal colonization. Depending upon the nature of root exudates, co-existing plants increase or decrease mycorrhizal colonization in the roots of each other (Javaid 2007; 2008).

Table 1. Effect of soil amendments, weeds and Biopower application on mycorrhizal colonization of rice 50 days after transplantation.

Treatments	Mycelial colonization (%)		Arbuscular colonization (%)		Vesicular colonization (%)	
	BP ⁻	BP ⁺	BP ⁻	BP ⁺	BP ⁻	BP ⁺
Recommended dose of N fertilizer						
Control (weed free)	27 a	27 a	13 a	13 b	7 a	7 a
<i>Echinochloa colonum</i>	30 a	23 a	20 a	20 ab	10 a	7 a
<i>Cyperus rotundus</i>	43 a	30 a	27 a	27 ab	10 a	7 a
<i>Marsilea minuta</i>	33 a	30 a	13 a	30 a	7 a	1 a
<i>Paspalum paspaloides</i>	20 a	33 a	23 a	30 a	7 a	7 a
Mixed weeds	43 a	47 a	30 a	33 a	13 a	13 a
Half dose of N fertilizer						
Control (weed free)	27 ab	20 b	17 b	10 c	1 b	10 a
<i>Echinochloa colonum</i>	33 ab	23 b	20 b	20 bc	7 ab	10 a
<i>Cyperus rotundus</i>	30 ab	33 ab	23 ab	27 a-c	7 ab	7 a
<i>Marsilea minuta</i>	17 b	20 b	17 b	17 bc	17 a	1 b
<i>Paspalum paspaloides</i>	33 ab	37 ab	33 ab	37 ab	7 ab	17 a
Mixed weeds	43 a	47 a	43 a	47 a	3 b	1 b
Farmyard manure						
Control (weed free)	17 a	13 a	17 a	13 b	10 a	10 b
<i>Echinochloa colonum</i>	20 a	23 a	20 a	23 ab	13 a	20 ab
<i>Cyperus rotundus</i>	20 a	20 a	20 a	20 ab	1 a	7 b
<i>Marsilea minuta</i>	17 a	13 a	17 a	13 b	3 a	10 b
<i>Paspalum paspaloides</i>	20 a	13 a	20 a	13 b	7 a	10 b
Mixed weeds	27 a	30 a	27 a	30 a	17 a	23 a

BP⁻ = Biopower absent

BP⁺ = Biopower present

In each soil amendment, values with different letters in a column show significant difference ($P \leq 0.05$) as determined by DMR Test.

There were insignificant differences between two corresponding treatments with and without Biopower application as determined by t-test.

Table 2. Effect of soil amendments, weeds and Biopower application on mycorrhizal colonization of rice 80 days after transplantation.

Treatments	Mycelial colonization (%)		Arbuscular colonization (%)		Vesicular colonization (%)	
	BP ⁻	BP ⁺	BP ⁻	BP ⁺	BP ⁻	BP ⁺
Recommended dose of N fertilizer						
Control (weed free)	47 a	50 a	40 a	30 a	23 ab	10 b
<i>Echinochloa colonom</i>	47 a	50 a	37 a	40 a	20 b	20 ab
<i>Cyperus rotundus</i>	40 a	50 a	40 a	50 a	10 b	27 ab
<i>Marsilea minuta</i>	50 a	37 a	40 a	37 a	27 ab	16 b
<i>Paspalum paspaloides</i>	53 a	60 a*	43 a	57 a*	33 ab	40 a
Mixed weeds	63 a	67 a	50 a	57 a	50 a	17 b
Half dose of N fertilizer						
Control (weed free)	47 a	30 a	43 a	30 a	47 a	37 a
<i>Echinochloa colonom</i>	50 a	57 a	47 a	53 a	40 ab	20 ab
<i>Cyperus rotundus</i>	37 a	40 a	37 a	40 a	30 ab	33 ab
<i>Marsilea minuta</i>	50 a	40 a	50 a	37 a	17 b	17 b
<i>Paspalum paspaloides</i>	60 a	63 a	57 a	63 a**	40 ab	30 ab
Mixed weeds	60 a	60 a	60 a	60 a	20 b	17 b
Farmyard manure						
Control (weed free)	27 a	40a*	27 a	40 a*	13 a	20 a
<i>Echinochloa colonom</i>	43 a	50 a	43 a	50 a	27 a	33 a
<i>Cyperus rotundus</i>	47 a	40 a	47 a	40 a	16 a	27 a
<i>Marsilea minuta</i>	47 a	50 a	47 a	50 a	13 a	30 a
<i>Paspalum paspaloides</i>	56 a	50 a	57 a	50 a	23 a	33 a
Mixed weeds	53 a	53 a	53 a	47 a	37 a	33 a

BP⁻ = Biopower absentBP⁺ = Biopower present

In each soil amendment, values with different letters in a column show significant difference ($P \leq 0.05$) as determined by DMR Test. *, **, Show significant difference between two corresponding treatments with and without Biopower application, at 5 and 1 level of significance, respectively, as determined by t-test.

Effect of Biopower on mycorrhizal colonization in rice:

The effect of Biopower application on colonization of various structures of VAM was insignificant in all the three soil amendment systems after 80 days of rice transplantation. However, at later growth stages mycorrhizal colonization showed a little and variable response to Biopower application. The response was generally varied with the weed species and soil amendment. In half and recommended doses of N doses, mycelial and arbuscular colonization were significantly enhanced by Biopower application in rice plants cultivated in mixed culture with *P. paspaloides*. Likewise, in recommended dose of N fertilizer and farmyard manure, Biopower application significantly enhanced mycorrhizal colonization in rice plants co-cultivated with *M. minuta* at final growth stage. Similar effect has also been recorded in weed free treatment in farmyard manure amendment after 80 days of rice transplantation. Results of this study are supported by finding of many earlier workers who have reported increased mycorrhizal colonization due to

application of beneficial microorganisms (Javaid, 2010; Oancea *et al.*, 2010). Biaciotto and Bonfante (2002) found that some strains of *Azospirillum* and *Paenibacillus* stimulated the growth of vesicular-arbuscular mycorrhizal formation. There is possibility that microorganisms produced some stimulatory substances like auxin, cytokinins, and gibberellins that had an effect on the morphology and physiology of root resulting in qualitative and quantitative alteration of the root exudates, with direct effects on development of mycorrhizal fungi (Tiberius and Cătălin, 2011). The present study also reveals that mycorrhizal response of a plant species to beneficial microorganisms varies with soil amendment and the plant species associated with the test crop.

The present study concludes that mycorrhizal colonization in rice is markedly enhanced due to mixed weeds in different soil amendment systems. Furthermore, the effect of beneficial microorganisms on mycorrhizal colonization in rice varies with soil amendment and the associated weed species.

Table 3. Effect of soil amendments, weeds and Biopower application on mycorrhizal colonization of rice 110 days after transplantation.

Treatments	Mycelial colonization (%)		Arbuscular colonization (%)		Vesicular colonization (%)	
	BP ⁻	BP ⁺	BP ⁻	BP ⁺	BP ⁻	BP ⁺
Recommended dose of N fertilizer						
Control (weed free)	20 a	23 a	20 a	20 a	7 a	3 a
<i>Echinochloa colonum</i>	17 a	27 a	10 a	13 a	13 a	17 ab
<i>Cyperus rotundus</i>	23 a	20 a	13 a	10 a	10 a	10 ab
<i>Marsilea minuta</i>	17 a	33 a*	17 a	33 a*	3 a	23 a
<i>Paspalum paspaloides</i>	17 a	20 a	17 a	20 a	13 a	17 ab
Mixed weeds	13 a	17 a	13 a	17 a	7 a	10 ab
Half dose of N fertilizer						
Control (weed free)	20 a	13 a	20 a	13 ab	20 a	23 a
<i>Echinochloa colonum</i>	10 a	17 a	10 a	17 ab	17 a	13 a
<i>Cyperus rotundus</i>	30 a	23 a	30 a	23 ab	13 a	17 a
<i>Marsilea minuta</i>	17 a	17 a	17 a	17 ab	13 a	13 a
<i>Paspalum paspaloides</i>	20 a	33 a*	20 a	33 a*	17 a	20 a
Mixed weeds	13 a	10 a	13 a	10 b	27 a	30 a
Farmyard manure						
Control (weed free)	17 a	20 a	10 b	20 b	17 b	17 b
<i>Echinochloa colonum</i>	33 a	30 ab	33 a	30 ab	33 ab	27 ab
<i>Cyperus rotundus</i>	23 a	30 ab	27 ab	30 ab	23 ab	23 ab
<i>Marsilea minuta</i>	20 a	40 a*	20 ab	27 ab	17 b	40 a*
<i>Paspalum paspaloides</i>	37 a	43 a	30 ab	33 ab	37 ab	43 a
Mixed weeds	43 a	40 a	40 a	40 a	43 a	37 ab

BP⁻ = Biopower absentBP⁺ = Biopower presentIn each soil amendment, values with different letters in a column show significant difference ($P \leq 0.05$) as determined by DMR Test.

*, Show significant difference between two corresponding treatments with and without Biopower application, at 5% level of significance as determined by t-test.

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