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# Influence of Different Levels of Phosphorus on the Effectiveness of Arbuscular Mycorrhizae in Rice Rhizosphere

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**A B S T R A C T**

Arbuscular Mycorrhyzae Fungal (AMF) spores were obtained through mass multiplication in maize (in a previous experiment) as a host crop. A study was conducted to test the effectiveness of AMF in rice as host, with three levels of $P_2O_5$ (0, 20 and 40 kg/ha) under submerged condition. By reducing levels of $P_2O_5$ to 50 % (20 kg/ha) comparable plant biomasses were recorded ($P < 0.05$) under AMF inoculation with that of highest level of $P_2O_5$ (40 kg/ha) with or without AMF inoculation. By reducing the P supply (20 kg/ha), measurable effect of mycorrhyzae ($P < 0.05$) was obtained for P and N concentration (1.27 and 7.88mg/g respectively) and their uptake (20.63 and 127.50 mg/plant respectively) in rice plants which were comparable with un-inoculated plants at 40 kg $P_2O_5$/ha. AMF spores and root colonization were found at all levels of P supply, although overall the numbers of mycorrhyzal spores and per cent root colonization decreased with increasing P under mycorrhyza inoculation which implies that saturation or inundation does not necessarily prevent the development of AMF association.

## Introduction

Arbuscular mycorrhizal fungi (AMF) are soil inhabitants belonging to phylum, Glomeromycota (Sturmer, 2012) which forms symbiotic association with plants. The association allows an alternative nutrient assimilation pathway for plants through its special structures *viz*: extraradical and intraradical hyphae, arbuscules and the root apoplast interface. Hyphae of AMF can extend beyond the root surface i.e. extend beyond the rhizosphere nutrient-depletion zones which allows AMF to access a larger volume of soil (Smith and Read, 2008) to mobilise plant nutrients.

AMF provide benefits to their host plant in many ways, the most important being the enhanced uptake of P from soil, both in upland and in wet land situation (Stevens *et al.,* 2002; Smith and Read, 2008; Smith and Smith, 2011). Koide and his co-workers in 2000 suggested that AMF plays a significant role in

crop P nutrition by increasing total uptake and in some cases increase the P use efficiency, which may be associated with increased plant growth and yield. If the fungal colonisation is disrupted or hampered, uptake of P, growth and in some cases yield can be significantly reduced (Thompson, 1994). Besides improved P nutrition by AMF (Smith and Smith, 2011), it was reported that mycorrhizal plants can transfer N to the host plants, ranging from 20 to 74% of the total N uptake (Tanaka and Yano, 2005; Fellbaum *et al.*, 2014) from both inorganic and organic N sources (Hodge *et al.*, 2001; Leigh *et al.*, 2009).

According to Kahiluoto and his associates in 2009, contributions of AMF to soil quality and functional capacity of soil are particularly favoured in low-input systems as compared to conventional cropping systems. In cases of high concentration of available soil P crops fail to respond to colonisation by native AMF as colonisation of roots by AMF is often suppressed.

When strong colonisation occurs under conditions of high soil P concentrations, crop growth may be reduced. With a high rate of AMF colonization in soil where P availability is low, more P can be accessed from the soil for plants through absorption from an extensive hyphal network, thereby increasing potential uptake of other nutrients.

The importance and function of AMF associations in upland rice are well documented (Maiti *et al.*, 2005, 2011), but not to a greater extent in case of aquatic or semi-aquatic systems. However, benefits on maintaining the AMF association under wetland conditions have been suggested for rice (Solaiman and Hirata, 1997; Purakayastha and Chhonkar, 2001). These beneficial effects of AMF in aquatic and semiaquatic environments suggest some parallels with terrestrial systems. In the present study, the effectiveness of AMF was tested in rice (Variety: Basundhara) with different levels of P fertilization under submerged situation.

## Materials and Methods

Surface field soil samples (0-15cm) growing rice crop was collected from the Instructional Cum Research (ICR) farm of the Assam Agricultural University for the present study. The soils were pulverised, mixed uniformly and air dried. The initial soil chemical parameters were analysed and shown in (Table 1).

## The treatment details

Three levels of $P_2O_5$ (0, 20 and 40 kg/ha) with ($M_1$) and without (Mo) AMF inoculation were arranged in four replications for the pot experiment. The recommended N (40 kg/ha) and $K_2O$ (20 kg/ha) were applied in all the treatments. Rice (Variety: Basundhara) was taken as the test crop. The experiment was laid out in a Completely Randomized Design (CRD).

## Planting of rice seeds

Rice seeds were germinated in water agar. The germinated radicles were inoculated with consortia of *Glomus* and *Gigaspora* spp. belonging to AMF @ 50 spore/seedling. The pots with $M_o$ treatment were pre inoculated with chopped maize roots and adhering soils (100 g/pot). The pots were kept at saturated conditions for 60 days.

## Shoot and root biomass

After 60 days of growth, the plants were uprooted and the fresh biomass of shoots and roots were determined. Portions (50 %) of the fresh roots were kept for colonization study. The remaining shoots and roots were dried in hot air oven at $105^0$C for 24 h for determination of dry matter.

## Isolation of AMF spores and per cent root colonization

Isolation of AM Fungal spores was carried out by wet sieving and decanting method of Gerdemann and Nicolson (1963) by using the sieves of sizes ranging from 710 µ to 53 µ. 100g fresh rhizosphere soil sample was mixed in 300 mL of distilled water and shaken in a mechanical shaker at 200 rpm for 24 h. The spores and spore clusters retained in 53 µ and 100 µ were washed and collected in a filter paper. The spores were separated under inverted steriozoom microscope based on their morphology. Spore abundance was expressed as the number of AM Fungal spores per 100 gram soil. The spores were then preserved in ringer solution in small vials for further characterization.

Per cent root colonization was accessed using the method of Phillips and Haymen (1970). Root samples were washed under tap water, cut into small pieces of approximately 1cm and boiled in 10 % KOH as a clearing agent for one hour. The boiled samples were acidified with 2 % HCl for five minutes and subsequently warmed in 0.05 % trypan blue in lacto-phenol. After pouring off the stain, lacto-phenol was added and kept overnight to de-stain the host tissues. Slides were prepared and examined under microscope for per cent root colonization

## Plant tissue analysis

Plant analysis is the quantitative determination of the concentration of an element or extractable fraction of an element in a sample from a particular part or portion of a crop. In the present study, concentration of two elements *viz*. nitrogen (N) and phosphorus were determined**.** The total N of the plant tissues were determined by Kjeldahl method as described by Johann Kjeldahl (1883). The total P of the plant tissues were determined by Vanadomolybdate method as described by Watanabe and Olsen (1965). The uptake of nutrients was calculated by multiplying the concentration of the nutrients in the tissue samples with the corresponding yields of shoot dry weight.

Nutrients Uptake (mg/plant) = shoot dry wt. × nutrient concentration

## Available phosphorus

The available $P_2O_5$ of the soil samples were determined using Bray's-I method meant for acidic soils (Bray and Kurtz, 1956; Jackson, 1973).

## Enzyme phosphomonoesterase activity

The method of Tabatabai and Bremner (1969) was followed to estimate Phosphomonoesterase (PHM) activity. It involves the use of an artificial substrate called *p*-nitrophenyl phosphate (*p*-NPP). The product of PHM activity, *p*-nitrophenol, is a yellow chromophore under alkaline conditions were detected colorimetrically and expressed as µg *p*-nitrophenol/g dry soil/hr.

## Statistical analysis

The experimental data obtained from various observations were analysed statistically by using analysis of variance in complete randomized block design (Pot experiment) as described by Gomez and Gomez (1984). Significance or non significance of the variance due to various treatments effect was determined by calculating respective 'F' values.

The standard error of mean was calculated by using the formula:

$$S.\,Em = \sqrt{\frac{EMS}{r}}$$

The critical difference (C.D.) at 5% probability level was calculated to find out the

mean difference between the treatments. CD was calculated by using the following expression.

C.D. = S. Em × value of t (5%) for error d.f. $\times\sqrt{2}$

Where,

t = table value of 't' at 5% and 1% probability level for error degrees of freedom.

To validate the effectiveness among the different variables, correlation coefficient and linear regression was used and the best fit was graphically represented (Fig. 3) as scatter diagram.

## Results and Discussion

### Effectiveness of mass multiplied mycorrhyzal spores in rice

The pot experiment as described in the materials and methods was carried out to understand the effectiveness of the mass multiplied AMF spore in rice crop with different levels of $P_2O_5$. The salient findings of the pot experiment are described below.

### Shoot and root biomass as influenced by levels of $P_2O_5$ and AMF inoculation in pot experiment

The shoot and root biomass as affected by levels of $P_2O_5$ and AMF inoculation are presented in Table 2. With increasing levels of $P_2O_5$ in the treatments the fresh shoot and root biomass increased significantly with or without AMF inoculation. The result showed, significantly highest fresh shoot (48.92 g/plant) and root (36.86 g/plant) biomass were recorded in the treatment $T_6$ that received highest amount of $P_2O_5$ (40 kg/ha) under AMF inoculation. The treatment $T_5$ received only 50% of $P_2O_5$ could produce comparable fresh shoot (41.81 g/plant) and root (31.21 g/plant) biomass with that of treatment $T_6$. However, the fresh shoot (44.15 g/plant) and root (28.92 g/plant) biomass in the treatment $T_3$ without AMF inoculation were comparable with the treatments $T_5$ and $T_6$. With reference to the dry matter, significantly highest shoot (18.93 g/plant) and root (14.94 g/plant) biomass were recorded in treatment $T_6$ followed by 17.61 g/plant and 12.03 g/plant in $T_3$ with and without AMF inoculation respectively. By reducing the $P_2O_5$ (20 kg/ha) in treatment $T_5$, the AMF inoculation however produced comparable shoot (16.25 g/plant) and root (13.48 g/plant) dry biomass with that of treatment $T_3$.

### Phosphorus and Nitrogen concentration and their uptake in rice plants as influenced by levels of $P_2O_5$ and AMF inoculation in pot experiment

The phosphorus (P) and nitrogen (N) concentration and their uptake in rice plant as influenced by levels of $P_2O_5$ and AMF inoculation are depicted in Figure 1 and 2 respectively. It was observed that with increasing levels of $P_2O_5$ both the P and N concentrations and their uptake were increased, but the magnitudes were more under AMF inoculation. Significantly highest concentration of P (1.33 mg/g) and N (8.25 mg/g) were recorded in the treatment $T_6$ that received highest amount of $P_2O_5$ (40 kg/ha) and AMF inoculation compared to the treatments $T_1$, $T_2$ and $T_4$ Figure 1. Similarly, in treatment $T_3$, under highest $P_2O_5$ application (40 kg/ha) could increase the P (1.28 mg/g) and N (7.98 mg/g) concentration which were at par with treatment $T_6$, but without AMF inoculation. But in the treatment $T_5$, by slashing the $P_2O_5$ (20 kg/ha) application under AMF inoculation, statistically comparable P (1.27mg/g) and N (7.88 mg/g) concentrations were obtained with that of treatments $T_3$ and $T_6$. Similar trends were observed in case of P and N uptake in rice plants. Under AMF inoculation and highest

application of $P_2O_5$ (40 kg/ha), the uptake of P (25.17 mg/plant) and N (155.06 mg/plant) in the treatment $T_6$ was significantly highest compared to the treatments $T_1$, $T_2$, $T_4$ and $T_5$ Figure 2. In the treatment $T_3$, though no AMF inoculation, under highest amount of $P_2O_5$ (40 kg/ha) application the uptake of P (22.61 mg/plant) and N (140.64 mg/plant) were at par with the treatment $T_6$. However, by reducing the $P_2O_5$ (20 kg/ha) under AMF inoculation in the treatment T5, the uptake of P (20.63 mg/plant) and N (127.50 mg/plant)were remain at par with the treatment $T_3$.

## Spore abundance, root colonization, available $P_2O_5$ and phosphomonoesterase activity as influenced by levels of $P_2O_5$ and AMF inoculation in pot experiment

The AMF spore counts and root colonization as affected by levels of $P_2O_5$ with and without AMF inoculation in the pot experiment are presented in Table 3.

In the experiment, significantly highest AMF spore counts of 487.50/100g soil were observed in the treatment $T_5$ that received 50 % of $P_2O_5$(20 kg/ha) with AMF inoculation. The treatment $T_6$ that received highest amount of $P_2O_5$ (40 kg/ha) could produce AMF spore of 225.00/100g soil even under AMF inoculation. Under lowest level of $P_2O_5$(0 kg/ha), the inoculation of AMF spore produces significant numbers of AMF spore (379.75/100g soil) in treatment $T_4$ compared to the non inoculated treatments($T_1$, $T_2$ and $T_3$).

Similarly, the root colonization were also found to be significantly higher in the treatments $T_5$ (56.57 %) and $T_4$ (47.50 %) under medium (20 kg/ha) and lowest (0 kg/ha) levels of $P_2O_5$ application respectively with AMF inoculation compared to non inoculation counterparts ($T_2$ and $T_1$). At highest level (40 kg/ha) of $P_2O_5$ application no significant differences of root colonization in the rice root was observed in the treatments $T_3$ (20.62 %) and $T_6$ (25.88 %) though the treatment $T_6$ received AMF inoculation.

The available $P_2O_5$ content in the rhizosphere soil of rice plants were increased with increasing levels of $P_2O_5$ application. Significantly higher amount of available $P_2O_5$ was observed in the treatments $T_3$ (12.20 ppm) and $T_6$ (11.02 ppm) compared to the treatments $T_2$ (9.36 ppm) and $T_5$ (9.17 ppm) that received 40 kg/ha and 20 kg/ha of $P_2O_5$ application correspondingly.

The phosphomonoesterase **(**PHM) activity in the rice rhizosphere was also influenced by application of different levels of $P_2O_5$.

In the present investigation, with increasing levels of $P_2O_5$ application the PHM also appeared to increase and significantly higher amount of PHM were observed in the treatment $T_3$ (274.62 µg *p* nitrophenol/g/h) and $T_6$ (280.74 µg *p* nitrophenol/g/h) compared to the treatments $T_1$ (185.91 µg *p* nitrophenol/g/h) and $T_4$ (195.55 µg *p* nitrophenol/g/h) respectively.

The linear regression line drawn between the PHM activities and available $P_2O_5$ in the rice rhizosphere [$y = 0.028 x + 2.912$ ($R^2 = 0.452$)] however displayed the significant positive correlation (r= 0.673**, n=18) (Fig. 3).

## Shoot and root biomass as influenced by levels of $P_2O_5$ and AMF inoculation in pot experiment

The results illustrated that there were no detectable differences ($P < 0.05$) between AMF inoculation ($T_6$) and without inoculation ($T_3$) treatments at 40 kg $P_2O_5$/ha level of P supply in fresh and dry shoot and root fresh weight of rice, although, in general, all increased with increasing P supply up to 40 kg $P_2O_5$/ha.

**Table.1** Initial status of soil chemical and biological parameters

| OC% | Ph | Av. N | Av. $P_2O_5$ | Av. $K_2O$ | Phosphomonoesterase (µg *p*-nitrophenol/g/h) |
|---|---|---|---|---|---|
| | | (ppm) | | | |
| **0.86** | 5.20 | 150.32 | 8.45 | 103.12 | 178.25 |

**Table.2** Effect of levels of $P_2O_5$ and AMF inoculation on shoots and root biomass

| Treatments | Shoot | Root | Shoot | Root |
|---|---|---|---|---|
| | **Fresh weight(gm/plant)** | | **Dry weight (gm/plant)** | |
| **$T_1$:$M_0N_{40}P_0K_{20}$** | 20.97 | 12.99 | 7.85 | 6.15 |
| **$T_2$:$M_0N_{40}P_{20}K_{20}$** | 30.18 | 17.39 | 12.95 | 8.71 |
| **$T_3$:$M_0N_{40}P_{40}K_{20}$** | 44.15 | 28.92 | 17.61 | 12.03 |
| **$T_4$:$M_1N_{40}P_0K_{20}$** | 23.08 | 15.81 | 10.49 | 7.81 |
| **$T_5$:$M_1N_{40}P_{20}K_{20}$** | 41.81 | 31.21 | 16.25 | 13.48 |
| **$T_6$:$M_1N_{40}P_{40}K_{20}$** | 48.92 | 36.86 | 18.93 | 14.94 |
| CD(0.05) | **6.31** | **4.41** | **2.54** | **1.97** |
| CV% | **12.19** | **12.45** | **12.21** | **12.63** |

**Table.3** AMF Spore, root colonization, available $P_2O_5$ and phosphomonoesterase activity as influenced by AMF inoculation

| Treatments | AMF Spore/100g soil | Root Colonization (%) | Av.$P_2O_5$ (ppm) | Phosphomonoesterase activity (µg *p* nitrophenol/g/h) |
|---|---|---|---|---|
| **$T_1$:$M_0N_{40}P_0K_{20}$** | 93.75 | 18.6 | 7.25 | 185.91 |
| **$T_2$:$M0N_{40}P_{20}K_{20}$** | 96.75 | 18.50 | 9.36 | 190.01 |
| **$T_3$:$M_0N_{40}P_{40}K_{20}$** | 84.00 | 20.62 | 12.20 | 274.62 |
| **$T_4$:$M_1N_{40}P_0K_{20}$** | 379.75 | 47.50 | 8.16 | 195.55 |
| **$T_5$:$M_1N_{40}P_{20}K_{20}$** | 487.50 | 56.57 | 9.17 | 258.07 |
| **$T_6$:$M_1N_{40}P_{40}K_{20}$** | 225.00 | 25.88 | 11.02 | 280.74 |
| CD(0.05) | **47.90** | **5.75** | **1.94** | **40.04** |
| CV% | **14.15** | **12.38** | **13.71** | **11.68** |

**Fig.1** Phosphorus (P) and nitrogen (N) concentration in rice plants as influenced by levels of $P_2O_5$ and AMF inoculation

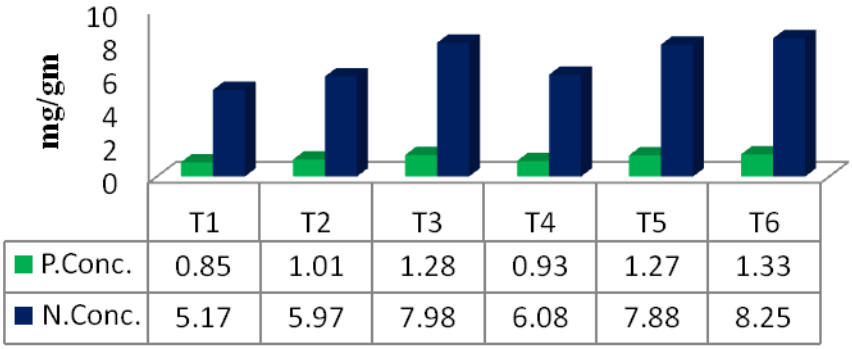



**Fig.2** Phosphorus (P) and nitrogen (N) uptake in rice plants as influenced by levels of $P_2O_5$ and AMF inoculation

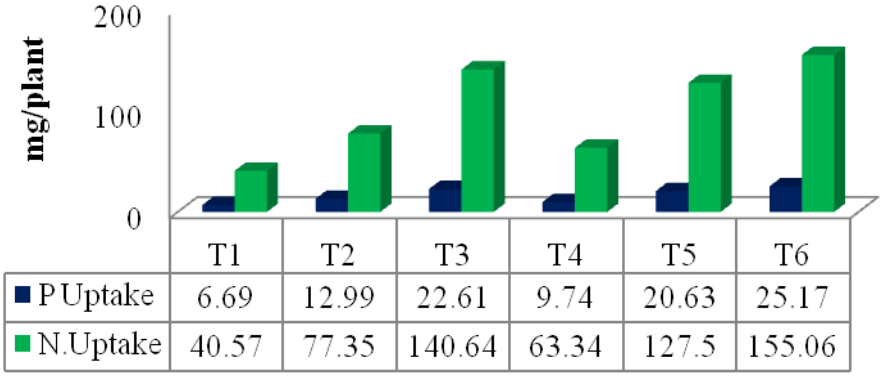
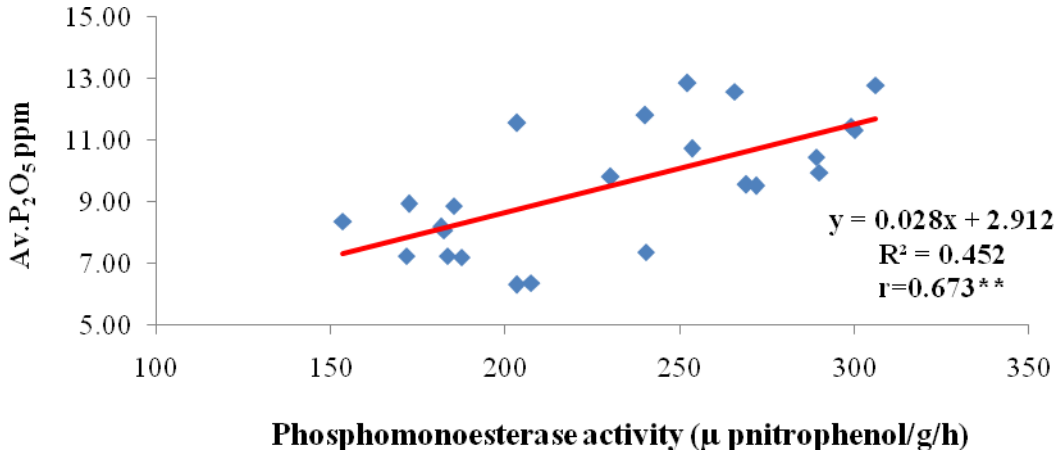



**Fig.3** Relationship between available $P_2O_5$ and phosphomonoesterase activity in rice rhizosphere



However, significantly comparable results ($P < 0.05$) for fresh and dry weight of shoot and root of rice ($T_6$ and $T_3$) were obtained by reducing the levels $P_2O_5$ of up to 50 % (20 kg/ha) in the treatment $T_5$ under AMF inoculation (Table 2). The distinctions in biomass (shoot and root) became evident 60 days following submergence and by the end un-inoculated plants ($T_3$) were similar with inoculated plants ($T_6$) at 40 kg $P_2O_5$/ha. However, the comparable plant biomass at 20 kg $P_2O_5$/ha ($T_5$) indicate that AMF have the potential to influence plant morphology in submerged soils and especially in the acidic soils of Assam.

## Phosphorus and nitrogen concentration and their uptake in rice plants as influenced by levels of $P_2O_5$ and AMF inoculation in pot experiment

When assessed in terms of P and N concentration and their uptake in rice plants ($P < 0.05$), the presence or absence of AMF did not lead to visible performance at highest level of P supply (40 kg/ha) in the treatments $T_6$ and $T_3$ respectively (Fig. 1 and 2). In fact, the only measurable effect of AMF on P and N concentration and their uptake in rice plants was obtained by reducing the P supply by 50% (20 kg/ha) in treatment $T_5$ which were comparable with un-inoculated plants ($T_3$) at 40 kg $P_2O_5$/ha (Fig. 1 and 2). AMF are naturally active under upland ecology and upland rice has been reported to be benefitted from AMF for P acquisition (Maiti *et al.*, 2005, 2011). In the acidic soils at very low levels of plant-available P, the medium P fertilization treatment ($T_5$) was probably the only treatment with adequate P supply for AMF dependence, and therefore, improved plant acquisitions of P and N was observed in this treatment under submerged situation. Besides improved P nutrition by AMF (Smith and Smith, 2011), it was reported that mycorrhizal plants can transfer N to the host plants, ranging from 20 to 74% of the total N uptake (Tanaka and Yano 2005; Fellbaum *et al.*, 2014) from both inorganic and organic N sources (Leigh *et al.*, 2009).

## Spore abundance, root colonization, available $P_2O_5$ and phosphomonoesterase activity as influenced by levels of $P_2O_5$ and AMF inoculation in pot experiment

AMF spores and root colonization were found at all levels of P supply, although in general the numbers of AMF spores and percent root colonization decreased with increasing P under AMF inoculation treatments ($T_4$, $T_5$ and $T_6$) (Table 3). This negative correlation between P availability and AMF spores and root colonization is well-documented in terrestrial systems (Smith and Read, 2008) and may also prevail in aquatic systems. Since P availability, is highly dependent on soil water content, the observed reductions in AMF and root colonization in submerged soils may be attributable to increased P availability rather than a direct result of increased water levels. In the current experiment the availability of P also significantly ($P < 0.05$) increased with increasing P supply and was found to be significantly correlated ($r = 0.673^{**}$) with the enzyme PHM (Table 3; Fig. 3). If the opposite were true, and AM colonization levels are dependent on water availability rather than P, colonization levels should remain constant under saturated conditions. This, however, was not the case in the current study and a similar reduction in root colonization levels with increasing P was found for *Lathrum salicaria* grown under saturated conditions (Stevens *et al.*, 2002) implies that saturation or inundation does not necessarily prevent the association from developing.

It can be concluded from the present investigation that by reducing the $P_2O_5$ level

by 50 % (20 kg/ha) along with recommended dose of N and $K_2O$, the effectiveness of arbuscular mycorrhyzae on enhancing plant biomass and nutrient uptake (P and N) in rice plant is comparable with the treatments of highest level of phosphatic fertilizer, with or with out the inoculation. Similarly, significantly highest AMF spore counts were observed in the treatment $T_5$ that received 50 % of $P_2O_5$ (20 kg/ha) along with AMF inoculation. Per cent root colonization were significantly higher in the treatments $T_5$ (56.57 %) and $T_4$ (47.50 %) under medium (20 kg/ha) and lowest (0 kg/ha) levels of $P_2O_5$ application respectively with AMF inoculation compared to non inoculation counterparts ($T_2$ and $T_1$). Elevated level of Phosphorus in soil does not necessitate high level of mycorrhyzal colonization. This study reveals that AMF works very efficiently under low and medium doses of phosphatic fertilizer in submerged conditions.

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