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Ornamental Plant Response to Phosphate Addition and Inoculation with Am Fungi and its Application in Restoring Polluted Soils

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Abstract Visakhapatnam is famously known as the "city of destiny". Rapid industrialization and urbanization have increased the levels of pollution in soils causing insignificant loss of ancient plant species and soil microflora.AM fungal association is present with almost all the plants. Residential and commercial area were taken into consideration and percentage AM colonization was identified. Two ornamental plant species were taken namely S. scutellarioides and I.herbistii. . Glomus sp and Acaulospora sp showed maximum colonization.There was increase in the levels of Phosphate with S. scutellarioides plant species and about 72% of colonization was seen in both the residential and commercial sites.The Phosphate levels were not upto the mark with I.herbstii species where colonization also resulted the same with about 32% in both the residential and commercial sites.

Keywords Arbuscular Mycorrhizal fungi, Ornamental Plants, Phosphate

1. Introduction

The limiting factor for plant growth is basically unavailability of nutrients which can be enriched by change in plant community. In generally, plants get adapted to infertile conditions in aged soils due to which plants become unable to survive. In turn, missing plant species can cause a decline in the abundance of soil organisms that require them as host (Perry et al.,1980), which has implication for the community and ecosystem processes that these soil organisms mediate. In restoring soils, this kind of mutualistic associations is essential for the functional restoration of disturbed soil systems (Harris et al.,2005; Perry and Amaranthus,1990; Renker et al.,2004; Whisenant,1999; Young et al.,2005) such as polluted soils.

Indiscriminate application of synthetic fertilizers not only causes detrimental environmental effects due to leaching, but also increases the global warming potential by 2% through their manufacturing process,which could be decreased markedly with nutrient recycling (Gilbert et al., 2011). Recycling nutrients from organic wastes, such as sludge, provides an opportunity to save energy and improve sustainability in agriculture (M.F. Seleiman et al., 2013).

Arbuscular Mycorrhizae, particularly play a fundamental role in development of vegetation in polluted soils. AM will also be limited by the availability of propagules (i.e. spores, hyphae, colonized roots) following the soil disturbance that occurs in the removal of vegetation for cultivation (Jasper et al., 1991; Johnson et al.,1991; Perry et al., 1989).

Arbuscular mycorrhizae (AM) contribute to plant growth and nutrient uptake, specially P (Smith and Gianinazzi-Pearson, 1988). Contribution can be a direct effect through an exploration of a larger volume of soil and/or a hyphal access to soil P pools not available to plants (Koide, 1991; Li et al., 1991; Jayachandran et al., 1992). Some authors even assert that AM mycelia may have P mineralizing activity (Jayachandran et al., 1992; Tarafdar and Marshner, 1994). Arbuscular mycorrhizal (AM) fungi are the ubiquitous in soil ecosystem and widespread symbioses occurring in terrestrial plant (Smith and Smith, 2011). Around 90% of land plants distributed in all major biomes are potentially mycorrhizal (Smith and Read, 2008; Smith et al., 2010; Smith and Smith, 2011). The remaining 10% of plants, including ***Chenopodiaceae*** and ***Brassicaceae***, are considered non-mycorrhizal. AM fungi can improve the nutritional status of the host plant through uptake of mineral nutrients, especially P, based on extensive mycelium in the soil (M.F. Seleiman et al.,2013).

It was predicted that the Phosphate fertilizer residues limit the enhancement of vegetation and mutual symbiosis of mycorrhizae with the plants of residential as well as commercial areas of Visakhapatnam, Andhra Pradesh. To test this prediction, a pot study was conducted in a green house with and without AM inoculums and response of the plants with Phosphate addition.

2. Materials and Methods

2.1. Experimental Design

This work was designed the experimental setup considering three factors: with and without AM inoculums (control) in residential and commercial sites, Phosphate addition and two ornamental plant species. Pots, each containing one plant were laid in the green house in two blocks as residential and commercial blocks for about two months.

The two native plants species selected were Solenostemon scutellarioides and Iresine herbistii . S.scutellarioides and I.herbistii are commonly known as Coleus and Blood leaf respectively. Though there are evidences that AM colonization in different Coleus plant species (Sailo and Bagyaraj, 2005) where no specific records of any fungal association is found in Bloodleaf plant as they belong to Amaranthaceae species. These plants were planted through the process of vegetation propagation.

In this study, soil samples were collected from the residential as well as commercial sites in and were placed in glasshouse for about 90 days and watered. A ratio of 1:3 ratio where one part was the collected soil samples and the rest three parts were sterilized sand soil (sterilized at 80^0C for1hour).pots of 8cm x 8cm x 15cm (depth).Additional plant nutrients along with Phosphates were given to both AM inoculated and uninoculated plants in the form of Hoagland's solution (absence of Phosphates) (Hoagland.1950).This solution was given to the plants for every 15 days and were hand watered (with deionised water) every day to prevent mycorrhizal contamination of uninoculated pots. Dead plants were replaced in the first two weeks.

2.2. Response Variables

The two plants species were harvested for 12 weeks and soil samples were collected randomly foe every 15^{th} day. All the physico-chemical and microbial parameters were analyzed(Table 1, Table 1.1, Table2 & Table2.1) .Soil pH, Nitrogen, Potassium, Phosphates and Organic Carbon were analyzed particularly as there was only negligible change in the values. Soil pH was measured using the pH meter ,Nitrogen was measured with Kjedahl apparatus, Phosphates and Potassium were measured using spectrometer. These values of pH, Nitrogen, Potassium, Phosphates and Organic Carbon were measured with interval of 15 days.

The soil samples were taken for every 15 days and the sporulation was assessed. 100g of soil sample was collected from the test pots for every 15 days, mixed with 1000ml of tap water and stirred well. After a minute they filtered in sieves arranged in descending order of Mesh size (250cm-37cm) order and filtered in filter paper . The filtered paper was viewed under stereolight microscope. After 12 week set up the fine roots of the plants were washed and sub sampled. The fine roots were then washed or cleared with 10% (w/v) Potassium Hydroxide at 55^0 C overnight and then stained 1% Trypan blue (v/v) Phillips and Hayman (1970) solution for 1 hour before being transferred to a solution of Lactoglycerol (Rodrigues, B, F., Muthukumar, T., 2009).

2.3. Statistical Analysis

Characterization of pH, Soil Organic Carbon, Total Nitrogen, Phosphates and Potassium were subjected to ANOVA using the Minitab 14 statistical package (Minitab, 2007).

3. Results

3.1. S.scutellarioides

There was no significant effect on the physico-chemical parameters of the test soil throughout the study period except with that of soil pH , Nitrogen, Potassium, Phosphates and Organic Carbon in both residential and commercial sites(Fig 1 & Fig 1.1). An increase in the levels of Phosphates was definitely seen in the test soil samples of both the residential and commercial soils along with a comparative increase in soil pH, Nitrogen, Potassium and decrease in Organic Carbon . The colonization of AM was also seen. The growth in the plant species was also identified as compared to that of control soils in all the test plants. Residential area where the colonization is more in ***S.scutellarioides*** in a range 40% to 70% as compared to the control soils or without AM fungi inoculums (Fig 3). Commercial zone showed a colonization percentage of 45% to 78% with control soils or without AM fungal innoculum which recorded less (Fig 3).

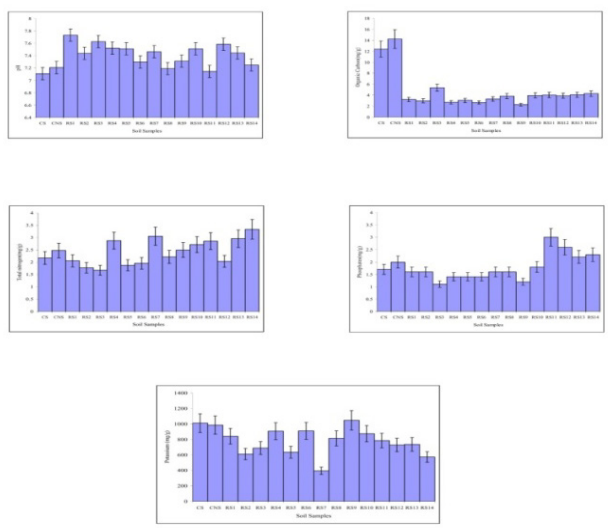

Figure 1. Statistical variation in pH, Organic Carbon, Total Nitrogen, Phosphates and Potassium in test soil samples of S. scutellarioides in Residential Zone

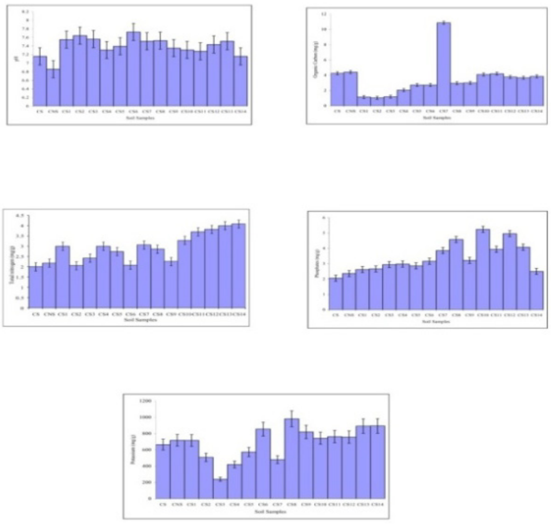

Figure 1.1. Statistical variation in pH, Organic Carbon, Total Nitrogen, Phosphates and Potassium in test soil samples of S. scutellarioides in Commercial Zone

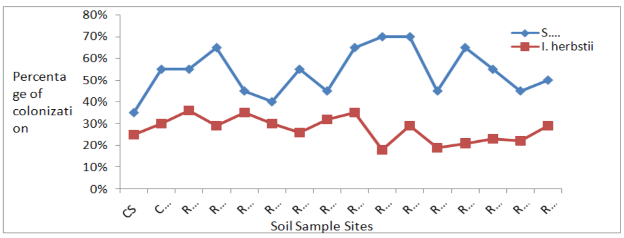

Figure 3. Percentage of fungal colonization in roots of rhizosphere soil of two test plants in Residential zone

3.2. I.herbistii

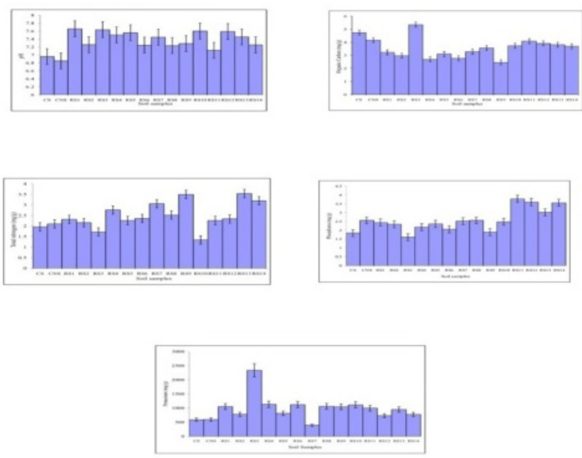

Figure 2. Statistical variation in pH, Organic Carbon, Total Nitrogen, Phosphates and Potassium in test soil samples of I.herbstii in Residential Zone

Though the test soil samples with this plant species also did not show any change in the physico-chemical parameters , there was even no increase in the Phosphates levels in the test soil samples of residential and commercial sites (Fig 2 & Fig 2.1). This plant species did not show any AM colonization throught the study period and the plant growth was also not significant. The colonization in ***I.herbstii*** was less in residential area with a array of 22% to 36% with comparison to control soil or without AM fungi inoculums (control) (Fig 4). Commercial zone showed a colonization percentage of 18% to 32% with control soils or without AM fungal innoculum with also recorded less with this plant species (Fig 4).

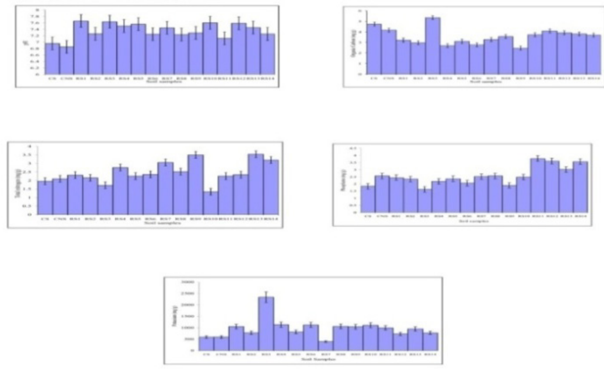

Figure 2.1. Statistical variation in pH, Organic Carbon, Total Nitrogen, Phosphates and Potassium in test soil samples of I.herbstii in Commercial Zone

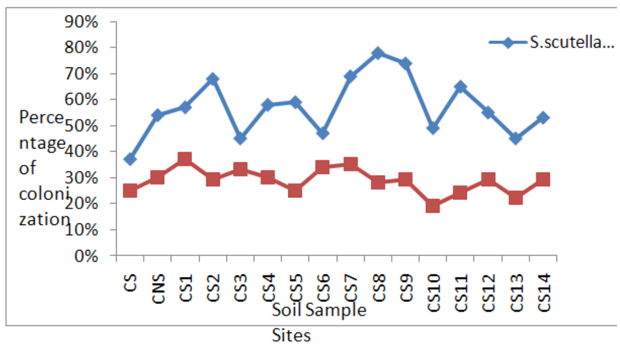

Figure 4. Percentage of fungal colonization in roots of rhizosphere soil of two test plants in Commercial zone

3.3.Colonization of the Test Soil Samples

Colonization of AM fungi varied from site to site and zone to zone. Though there was difference between the sampling sites but both the zones namely residential and commercial zone, the number spores of ***Glomus sp*** and ***Acaulospora sp*** were maximum and found were more in number with ***S.scutellarioides*** where ***I.herbstii*** showed very less spore count.

3.4. Morphology of the Test Plants

During the experimental work the plants grown represents the status of colonization and sporulation in the roots by AM fungi. The leaves of ***S.scutellarioides*** showed large surface area which represent the plant species had a good range of photosynthesis. ***S.scutellarioides*** showed good results in both zones where leaves of ***I.herbstii*** had very less surface area in both zones when compared with other test plants which showed decreased process of photosynthesis.

4. Discussion & Conclusion

Analysis physico-chemical and microbial parameters of the test soil samples of residential and commercial resulted in insufficiency of vital mineral nutrients like Nitrogen, Phosphate and Potassium. As focused by Kumar et al (2010) that in the process of AM root colonization pH factor plays a vivid role and in trivial acidic condition the pH shows no problem for the growth of the plant. The pH in soils which showed slightly acidic values were helpful for sporulation of Glomus sp where as acidic pH showed the sporulation of Acaluspora sp as studied by Porter et al (1987), Wang et al (1993) and Gia and Liu (2003).

The concentration of Nitrogen, Potassium and Phosphate was increased during the study period .Zhang et al (2003) suggested that a drop off soil pH, Electrical Conductivity, Organic Carbon and boosting in soil vital nutrients like Nitrogen, Potassium and Phosphate may be due to increased concentrations of Organic acids because of AM colonization. As stated by Neuman and George (2004) and Subramanian et al (2006) AMF spore production can be more or less on application of Phosphorous rich fertilizers. On the other hand Hui Jiao et al (2011) stated that there is a reduction in AMF sporulation on application of fertilizers.

In increased Phosphorous levels there is a decline in the spore number as well as root colonization due to less exudation (Ratnayake et al .,1978 and Graham et al., 1981).But the reduction in soil Phosphorous may be one of the reason for AM colonization which hamper the hyphal growth. Nitrogen results a decrease in the AM colonization but it helps to increase the number of spores in the rhizosphere area of the soil (Redhead, 1975).Muthukumar et al (1998) described that Potassium also showed a negative relation with the AM colonization with good sporulation in rhizosphere zone of the plant.

Among all the two plant species one of the species namely S.scutellarioides responded well and illustrated good amount of spores with effective root colonization whereas the second species as ***I. herbstii*** which belong to the family Amaranthaceae, having less or no capability to have bonding with AM fungi as stated by Sheraz Mahdi et al (2010).The above result suggested that the two plant species can be used for land restoration , in less fertilized soils etc as they have shown best colonization with good plant growth.

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