Interaction Studies Between Vam (*Glomus fasciculatum*) and Other Beneficial Microorgansims on Growth of Foxtail Millet (*Setaria italica* (L.) Beauv.) SiA-326 Variety

Dr. Laxman B Kadam[1]

[1]Assistant Professor, Department of Botany, K. R. C. E. S'S., G. G. D. Arts, B. M. P. Commerce and S. V. S. Science College, Bailhongal-591102, Karnataka, India
Corresponding Author: bandukadam017[at]gmail.com

Abstract: ***Mycorrhizal fungi plays an important role in soil micro biota and certain soil microorganisms are known to regulate mycorrhizal formation and function. Conversely, mycorrhizae affect the establishment of rhizosphere populations. Several interactions between mycorrhizae and soil microorganisms involves in nutrient cycling, hence having an impact on plant growth and nutrition. In the present study, similar findings have been recorded. An increased 'p' uptake was seen in the plants inoculated with VAM and Bacillus polymyxa. This might be due to the enhanced utility of sparingly soluble form of 'p' in the soil. The present investigation showed that the combined inoculation of VAM, Azospirillum and Bacillus polymyxa in selected foxtail millet SiA-326 variety is more advantageous in obtaining maximum growth and yield. Hence, an overall enhancement of growth and yield is observed when plants were inoculated with the combination of VAM, Azospirillum and Bacillus polymyxa.***

Keywords: Mycorrhizal, Microorganisms, *Bacillus polymyxa*, SiA-326 variety, *Azospirillum*, *Bacillus polymyxa*

1. Introduction

Mycorrhizal fungi plays an important role in soil micro biota and certain soil microorganisms are known to regulate mycorrhizal formation and function. Conversely, mycorrhizae affect the establishment of rhizosphere populations. Several interactions between mycorrhizae and soil microorganisms involves in nutrient cycling, hence having an impact on plant growth and nutrition. Other interactions are in concern to root pathogen activity, there by affecting biological control to benefit plant health. Interactions of VAM fungi with other soil organisms are well known phenomena, which have been studied for several decades. Many observations of similar interaction of VAM fungi with various fungi and different groups have been observed. It is now widely accepted that the soil microorganisms i.e. especially AM fungi and plant roots form an ecological important complex, dependent on energy and photosynthates supplied by the plant (Grayston *et al*., 1996). Plant roots release energy rich organic compounds through the roots as exudates. These root exudates may be about 25% of total assimilated carbon (Jones and Darrah, 1995) and up to 40% of dry matter produced by the plant (Lynch and Whipps, 1990). Such a massive efflux of energy rich compounds into the soil results in the formation of a specific zone around the root called the rhizosphere. This soil zone affected by colonized host root by mycorrhizae is called mycorrhizosphere (Linderman, 1992). The SiA-326 variety was selected for experiments.

2. Review of Literature

Hiltner coined the term rhizosphere in 1904 to denote the region of the soil subjected to the influence of plant roots and it is characterized by intense microbial activity. Many workers have shown that the microflora of the rhizosphere differs qualitatively and quantitatively from that microflora which is beyond the infuence of the root (Parkinson, 1967; Bagyaraj and Rangaswamy, 1972; Bowen and Rovira, 1976). The rhizosphere effect is greatest with bacteria followed by actinomycetes and fungi. The increased microbial activity in the rhizosphere has been attributed to extra nutrients available in that region. Bagyaraj and Menge (1978) reported that the larger populations of bacteria and actinomycetes were found in the rhizosphere of tomato plants inoculated with VAM fungus (*Glomus fasciculatum*) and Bacteria (*Azotobacter chroococcum).* Ames *et al.* (1984) tested the integration of mixture of bacteria and VAM fungus isolated from field-collected soil of *Bouteloua gracilis* in a pot culture trial using the same host. In the rhizosphere of mycorrhizal plants and the total bacterial population colony counts of the four bacterial isolates used in the study which was expressed as colony forming units (CFU) per gram of root dry weight. The population of the Actinomycetes in the mycorrhizal rhizosphere of pot grown *Bouteloua gracilis* plants was lesser when compared to control plants. But no negative correlation was found between the amounts of mycorrhiza formation with respect to actinomycete population. Studies on microbial interactions in the mycorrhizosphere have focused on exotic populations of beneficial soil microorganisms, such as associative N_2 fixing bacteria *Azosprillum* (Subba Rao *et al*., 1986), plant growth promoting rhizobacteria (Meyer and Linderman, 1986) and phosphate-solubilizing bacteria (Toro *et al*., 1996). Secilia and Bagyaraj (1987) estimated the population of total bacteria, nitrogen fixing bacteria and actinomycetes in the root zones of different VAM pot cultures, viz., *Glomus fasciculatum*, *Gigaspora margarita* and *Sclerocystis dussi.* They observed significantly greater population of total nitrogen fixing bacteria and actinomycetes in mycorrhizal pot cultures. Mycorrhizae may affect both the number and the composition of bacterial populations differently in their own rhizosphere and hyphosphere (Linderman, 1988). Azcon (1989) studied the tomato plant growth in presence of rhizosphere bacteria and VAM fungi *Glomus mossae* and *Glomus fasciculatum*. In general, bacterial inoculation increased the growth of mycorrhizal plants. Harinikumar *et al., (*1991) determined the effect of associative microflora on cowpea, singly and dually with *Glomus fasciculatum*. Among the associative micro flora studied, the gram-positive bacterium, *Cladosporium* had stimulatory effect on mycorrhizal colonization and sporulation, when they were inoculated along with VAM. However, associative microorganisms, when added alone to plant had no significant effect on plant growth. Krishnaraj and Sreenivas (1992) observed that the *Capsicum annuum* inoculated with VAM fungi harbored more number of bacteria in the endomycorrhizosphere than control. They also observed that bacterial population in the roots increased with increase in mycorrhizal root colonization. Nirmalnath and Sreenivas (1992) studied the effect of *Glomus fasciculatum* and phosphate solubilizing bacterium, *Pseudomonas striata* at four levels of two forms of 'P' (superphosphate and rockphosphate) on rhizosphere microflora of sunflower (*Helianthus annus* L.). They observed that the total bacteria, fungi and actinomycetes significantly increased with increase in the level of 'P' and with the age of the host plant.

Singh (1992) observed that the inoculation of nitrogen fixing bacteria (*Azospirillum brasilense, Azospirillum lipoferum* and *Azotobacter chroococcum*) and phosphate solubilizing bacteria (*Bacillus polymyxa* and *Pseudomonas striata*) enhanced sporulation, root volume and percent VAM root colonization of *Pennisetum padicillatum* in the presence of *Glomus macrocarpum*. The interaction effects of phosphate solubilizing bacterium (*Enterobacter agglomerans)* and VAM fungus (*Glomus etunicatum)* increased the growth of tomato plant (Kim *et al.*, 1998). Gaonker *et al., (*1993) reported that inoculation of *Glomus fasciculatum* in conjunction with different organic amendments having a narrow C: N ratio increased the population of rhizosphere bacteria, fungi, actinomycetes and free living nitrogen fixers in different genotypes of wheat up to 90 days. Srihari and Sreenivasa (1995) observed higher population of free-living nitrogen fixers and 'P' solubilizers in the rhizosphere of chilli when inoculated with *Glomus macrocarpum* and *Bacillus polymyxa* as compared to control.

Gurumurthy and Sreenivasa (1996) have found higher population of free-living N_2 fixers and 'P' solubilizers in the rhizosphere of chilli inoculated with *Glomus macrocarpum* compared to UIC. Redecker *et al., (*1997) studied the interaction between *Phaseolus vulgaris*, *Rhizobium* strains nodulating *Phaseolus vulgaris* and VAM fungi under green house condition using non-sterilized soil. The result implies that in low 'P' soil, the effect of an improved mycorrhizal symbiosis may include improved symbiotic N_2 fixation efficiency and improved N-uptake. The availability of 'P' in soil is intimately related to the parent materials present in the soil. 'P' mineralization and Solubilization of nutrients are microbial related processes, which can directly control 'P' availability (Kaupulnik and Douds Jr, 2000).

3. Materials and Methods

All experiments were conducted under poly house conditions. The physici-chemical properties were determined Jackson (1973) (Table 1.). The soil was sandy loamy, which was filled in earthen pots of 15 cm diameter in the ratio of 1: 1 (one parts soil+ one part of sand). Soil was steam sterilized and the seeds of Foxtail Millet varietiy SiA-326 were sown in the pots with the other microorganisms in different combination as follows,

1. Seeds sown in sterilized soil without inoculum (UIC)
2. Seeds + *Glomus fasciculatum (*15 g/pot, sporocarps + hyphae).
3. Seeds + *Glomus fasciculatum* + *Azotobacter* in the form of slurry.
4. Seeds + *Glomus fasciculatum* + Phosphate solubilizing bacteria (*Bacillus polymyxa)* in the form of slurry
5. Seeds +*Glomus fasciculatum* +*Azotobacter* +Phosphate solubilizing bacteria (*Bacillus polymyxa)* in the form of slurry.

Table 1: Physico-Chemical characteristics of Soil used for Experiments

Soil Type	**Potting Mixture Garden soil: sand (1: 1)**
Soil type	Sandy loamy
Soil moisture (%)	28.23
pH	6.67
Electric conductivity (M mhos/cm at 25 °C)	0.546
Organic carbon (%)	0.45
N (%)	1.23
P (%)	0.22
K (%)	2.48
Zn (%)	2.31
Cu (%)	1.01
Mg (%)	1.24

The treatments were set up in triplicates and maintained in poly-house conditions. The Foxtail Millet SiA-326 variety was inoculated with *Glomus fasciculatum*. The un-inoculated treatment served as the control. Pots were watered on alternate days to maintain moisture. 15 ml of Hoagland plant nutrient solution was given at every fifteen days interval from the day of sowing. Periodical data were recorded at different intervals of 20, 40 and 60 days. The

VAM colonization (Phillips and Hayman 1970), number of spore per 50g soil (Gerdemann and Nicolson, 1963) and nitrogen and phosphate in shoot was estimated as outlined by Jackson (1973).

4. Results and Observation

The present investigations have shown after 20 days (Table 2) the Foxtail Millet SiA-326 variety inoculated with VAM+ *Azotobacter* +*Bacillus polymyxa* significantly increased shoot height (29.63 cm), root length (7.56 cm), number of rootlets (6), number of leaves (6), per cent of colonization (57.99), spore number (145 spores / 50g soil), shoot dry weight (0.045g), root dry weight (0.008g), per cent of nitrogen uptake (1.39 %) and per cent of phosphorus uptake (0.21%) which was higher as compared to VAM+*Azotobacter*, VAM+PSB and UIC (Uninoculated control) (Table 2). But the Foxtail Millet inoculated with VAM and *Azotobacter* has higher "N" uptake (1.30%) than the plants inoculated with VAM and PSB (*Bacillus polymyxa) (*1.24%). However, dual inoculation of VAM and PSB (*Bacillus polymyxa*) showed higher 'P' uptake (0.18%) compared to combination of VAM and *Azotobacter* (0.14%).

The present investigations have shown that after 40 days (Table 2) the Foxtail Millet SiA-326 variety inoculated with VAM+ *Azotobacter* +*Bacillus polymyxa* significantly increased shoot height (64.50 cm), root length (27.83 cm), number of rootlets (13), number of leaves (7.66), per cent of colonization (75.47%), spore number (132.33 spores / 50g soil), shoot dry weight (0.38g), root dry weight (0.086g), per cent of nitrogen uptake (1.43 %) and per cent of phosphorus uptake (0.30%) which was higher as compared to VAM+*Azotobacter*, VAM+ PSB and UIC (Uninoculated control) But the Foxtail Millet inoculated with VAM and *Azotobacter* has higher "N" uptake (1.38%), than the plants inoculated with VAM and PSB (*Bacillus polymyxa) (*1.27%). However, dual inoculation of VAM and PSB (*Bacillus polymyxa*) showed higher 'P' uptake (0.27%), compared to combination of VAM and *Azotobacter* (0.24%).

Table 2: Interaction of *Glomus fasciculatum*, *Azotobacter* and PSB on Shoot length, root length, no of rootlets, no of leaves, % of colonization, spore no /50 g soil, shoot dry weight, root dry weight, shoot/root ratio, P uptake of Fxtail Millet (*Setaria italica* (L.) Beauv.) var-SiA-326

Treatment/duration	Plant length (cm)	Root Length (cm)	No. of Rootlets	No of Leaves	Colonization (%)	No of spores/50g soil	Dry weight of shoot (g)	Dry weight of root (g)	N uptake in shoot (%)	Phosphorus uptake in shoot (%)
20 DAYS										
UIC	15.10c	4.66d	4.00b	4.00b	0.00c	0.00c	0.013b	0.003b	1.03d	0.07e
M	19.73bc	5.30c	5.00ab	5.00ab	39.47b	70.33b	0.017b	0.004b	1.17c	0.11d
M+AZ	21.66b	5.46c	5.00ab	5.00ab	41.83b	86.33b	0.020b	0.005b	1.30b	0.14c
M+PSB	22.46b	6.00b	5.00ab	5.00ab	42.39b	94.33b	0.025b	0.006b	1.24bc	0.18b
M+AZ+ PSB	29.63a	7.56a	6.00a	6.00a	57.99a	145.00a	0.045a	0.008a	1.39a	0.21a
40 DAYS										
UIC	43.76e	14.20b	6.33b	3.66b	0.00c	0.00d	0.17d	0.014c	1.04d	0.11d
M	52.76d	18.03b	7.33ab	6.66b	59.48bc	87.66c	0.22cd	0.028b	1.17c	0.17c
M+AZ	56.63c	18.86b	8.66ab	6.66b	62.53b	102.00bc	0.28bc	0.034b	1.38a	0.24b
M+PSB	60.16b	20.30b	10.00ab	7.00a	68.39ab	124.00b	0.31ab	0.037b	1.27b	0.27ab
M+AZ+ PSB	64.50a	27.83a	13.00a	7.66a	75.47a	232.33a	0.38a	0.086a	1.43a	0.30a
60 Days										
UIC	54.76c	21.16c	9.00c	6.00c	0.00d	0.00c	0.31c	0.04b	1.14d	0.12e
M	79.93b	22.70bc	13.66b	7.33bc	75.43c	214.66b	0.82bc	0.12bc	1.25c	0.24d
M+AZ	84.60b	23.76bc	14.33b	7.33bc	76.43b	229.33b	0.94b	0.13bc	1.42b	0.28c
M+PSB	87.10b	26.20b	14.66b	8.00ab	80.16a	250.00b	1.07b	0.19b	1.31c	0.32b
M+AZ+ PSB	103.16a	35.56a	17.33a	9.00a	87.18a	292.33a	1.72a	0.34a	1.48a	0.37a

UIC-Uninoculated contrl, M=Mycorrhizal, Mycorrhizal (*Glomus fasciculatum*),
M+AZ=Mycorrhizal+*Azotobacter*, M+PSB=Mycorrhizal+Phosphate solubilizing bacteria,
M+AZ+ PSB= Mycorrhizal+*Azotobacter*+ Phosphate solubilizing bacteria.
*Mean values followed by the same letter within a column do not differ significantly at *P*=0.05 according to DMRT.

The present investigations have shown after 60 days (Table 2) that the Foxtail Millet SiA-326 variety inoculated with VAM+ *Azotobacter* +*Bacillus polymyxa* significantly increased shoot height (103.16 cm), root length (35.56 cm), number of rootlets (17.33), number of leaves (9), per cent of colonization (87.18%), spore number (292 spores / 50g soil), shoot dry weight (1.72g), root dry weight (0.34g), per cent of nitrogen uptake (1.48 %) and per cent of phosphorus uptake (0.37 %) which was higher as compared to VAM+*Azotobacter*, VAM+ PSB and UIC (Uninoculated control). But the Foxtail Millet inoculated with VAM and *Azotobacter* had higher "N" up take (1.42%), than the plants inoculated with VAM and PSB (*Bacillus polymyxa) (*1.31%). However, dual inoculation of VAM and PSB (*Bacillus polymyxa*) showed higher 'P' uptake (0.32%) compared to combination of VAM and *Azotobacter* (0.28%).

5. Discussion

The fast depletion of fossil fuel resources, increasing cost of chemical fertilizers and environmental pollution has drawn more attention to the use of microorganisms, with the aim of increasing the availability of nutrients to plants. The tripartite association of mycorrhizal fungi, nitrogen fixing organisms and phosphate solubilizing bacteria, has been a subject of interest in recent days. The various microorganisms associated with rhizosphere system, in which VAM, a fungus, occupies a unique ecological position. The internal phase of mycorrhizal fungus obtains a good source of nutrient from the host. Positive role of microorganisms in nutrition and growth of Foxtail Millet is established beyond doubt. This advantage enables them to have an intimate contact with roots and thus include their chances of exerting a greater effect on plant than other microbial species restricted only to the rhizosphere. The combined inoculation of VAM, *Azospirillum* and *Bacillus polymyxa* showed increased growth and yield, which is in accordance with Mohan Das (1987). In the present study, similar findings have been recorded. An increased 'P' uptake was seen in the plants inoculated with VAM and *Bacillus polymyxa*. This might be due to the enhanced utility of sparingly soluble form of 'P' in the soil. These findings are consistent with Barea *et al*., (1983). The present investigation showed that the combined inoculation of VAM, *Azospirillum* and *Bacillus polymyxa* in selected Foxtail millet SiA-326 variety is more advantageous in obtaining maximum growth and yield. Hence, an overall enhancement of growth and yield is observed when plants were inoculated with the combination of VAM, *Azospirillum* and *Bacillus polymyxa*. Similar findings are reported by earlier workers (Gurumurthy and Sreenivasa, 1996; Redecker *et al.,* 1997; Behl *et al*., 2003).

While exploring the merits of the direct oxidation pathway, it is time too, to emphasize certain variables that need to fit along with the mineral phosphate solubilization (MPS). The phenomenon of other important metabolic pathways of the mineralization of organic phosphates is the conversion of insoluble organic phosphate into ionic forms that can be taken up by the plants. This also happens in rhizosphere and its importance in increasing the bivarible Pi (inorganic phosphate) in the rhizosphere has not been worked upon (Lakshman, 1999 and Sen, 2005). Increased plant growth with cell elongation and multiplication due to enhanced nutrient uptake (in the present work P and N) by plants following inoculation of *Azospirillum* and 'P' solubilizing bacteria has probably caused the increased plant height (Black, 1968). In addition, the organisms are also known to produce certain growth promoting substances that influence plant height and the same is reflected with the plant dry matter accumulation in Foxtail Millet.

These pot trials are an important step for correct management of VAM and these trials must be designed to determine basic characteristics such as host–fungus specificity interaction and possible environmental stresses, such as drought stress, salinity, "N" and "P" deficiency conditions. The enhancement of microbial population in the combined inoculation of two or more organisms might be due to synergistic relation between the N_2 fixers, phosphate absorbers and 'P' solubilizers which helped in the multiplication of each other. The physiological promotion characteristics such as N_2 fixation, P-solubilization and phytohormone production may also play a very important role in plant growth (Hoflich *et al.*, 1994). Therefore, the present study concludes that PSB under specific conditions mobilizes unavailable forms of soil fertilizer or 'P' and VAM fungus *G. fasciculatum* provided soluble nutrients to plants, which improves plant nutrient uptake and growth.

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