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Effect of co-inoculation of AM fungi and two beneficial microorganisms on growth and nutrient uptake of *Eleusine coracana* Gaertn. (Finger millet).

Geeta. B. Patil[1], H. C. Lakshman[2], Romana. M. Mirdhe[2] and B. S. Agadi[1]

[1]*Department of Botany, P. C. Jabins Science College, Vidyanagar, Hubli, INDIA*
[2]*Post Graduate Department of Studies in Botany, Microbiology Laboratory, Karnatak University, Dharwad, INDIA*

ABSTRACT

Earthen pot experiments were carried out to study the co-inoculation effect of Arbuscular Mycorrhizal (AM) fungi (Glomus fasciculatum), Azospirillium brasilense and PSB on plant height, dry weight of root and shoot, per cent root colonization, spore number, P and N uptake. Experimental pots were filled with 4 kgs of sterilized soil and maintained in green house at 25-30^{o} C temperature. Single inoculation of AM fungi and combined inoculation of AM fungi with Azospirillium brasilense or PSB was found to be moderately increased in all the growth parameters. However triple inoculation of AM fungi, Azospirillium brasilense and PSB was found to have highest growth parameters.

Key words: *Glomus fasciculatum*, *Azospirillium brasilense*, per cent colonization, biomass production.

INTRODUCTION

Arbuscular Mycorrhizal (AM) fungi are found in most of the soils around the world, and they form association with 80% of all terrestrial plant roots [7]. The beneficial effects of AM fungi symbiotic association on the growth of plants are well known [21,13]. Arbuscular mycorrhizal fungi help in water regulation of plants by extending their hyphae towards the available moisture zone for continuous water absorption and translocating them to plants. Arbuscular mycorrhizal association can affect the host plants in terms of stomatal movement and photosynthesis of leaves and has been shown to increase the rate of transpiration, photosynthesis and chlorophyll content [15, 2]. Most land plants are symbiotic with arbuscular mycorrhizal fungi (AMF) and N_2 fixing bacteria, which taken up mineral nutrients from the soil and exchange them with plants photosynthetically fixed carbon.

Infact, the rhizosphere is a heterogeneous, continuous and natural habitat in which different types of interactions occur between soil microbes and plants. The beneficial plant microbe interaction in the rhizosphere is the primary determinants of plant health and soil fertility. Concentrated efforts are being made worldwide to develop nutrient use-efficient crop cultivars responsive to bio-fertilizers to increase crop yield and also to maintain soil good health. It is indicated that plants might select the AMF and plant growth promoting bacteria such as nitrogen fixers. The current emphasis is on sustainable agriculture, which uses less of chemical inputs like fertilizers and pesticides having adverse effect on soil health, fertility and environment [11]. Thus, use of microbial inoculants play an important role in sustainable agriculture. Arbuscular mycorrhizal fungi are known to improve the nutritional status, growth and development of plants, protect plant against root pathogens and offer drought resistance to drought and salinity [9].

Eleusine coracana Gaertn. (Finger millet) is commonly called as finger millet or African millet. This is the third important millet of India, originated from Africa, later migrated to south East Asia. The flour obtained by grinding the grain is used for preparing cakes, porridge and pudding etc. the grain is used for preparing fermented beverages,

such as beer. The stalks are used as fodder for milking cattle. Interaction of arbuscular mycorrhizal (AM) fungi with soil organisms is a well known phenomenon. Several studies in recent years have explored the interaction between AM Fungi and *rhizobium* on leguminous hosts [12]. However there is very less report on the interaction of AM fungi with other microorganisms such as *Azospirillium* and *azotobacter* in finger millet varieties that play a significant role in plant growth. The potential of these microbes as a biofertilizer has been investigated in the present work.

MATERIALS AND METHODS

The pot experiments were conducted in triplicate. Each pot measuring about 30cm in diameter were filled with soil and sand in the ratio of 2:1 which was sterilized by fumigating with 5% methyl bromide. The following treatments were given to the experimental pots.

1. Control
2. AM fungi
3. AM fungi+ *Azospirillium brasilense* (in the form of slurry)
4. AM fungi+ *Bacillus polymyxa* (PSB)(in the form of slurry)
5. AM fungi+ *Azospirillium brasilense* + *Bacillus polymyxa*(PSB)

Regular watering of experimental plants was done to maintain soil moisture. Periodical data on growth parameters were recorded at 30,60, 90 days interval. Parameters like plant height, dry weight of shoot and root, per cent root colonization. Nitrogen was determined by microkjeldal and phosphorous was determined by phosphoric acid yellow colour method [3]. Per cent of mycorrhizal colonization in the root was determined by root slide technique. After clearing the roots with 10% KOH and stained with 0.05% tryphan blue [17].

$$\text{Root colonization} = \frac{\text{Number of colonized segments}}{\text{Total number of segments examined}} \times 100$$

Number of spores in the soil surrounding the roots was determined by the wet sieving and decanting technique [5] and identified with the help of manual [18]. Data were analyzed using one way ANOVA.

Table 1. Showing physico-chemical properties of soil used for the pot experiments

Characteristics	Garden soil
pH	6.7
Soil moisture (%)	28.04
Organic matter (%)	0.82
E. C	0.97
N	1.42
P	0.27
K	2.42
Zn	2.03
Cu	1.06
Mg	1.43
Pb	0.95

RESULTS AND DISCUSSION

The results of soil analysis and the effects of different treatments of inoculam (single, dual and triple) have been presented in Table 2. In the present investigation combined inoculation of AM Fungi with *Azosprillium* and *Azotobacter* significantly increased all the growth parameters and yield in the finger millet. There was also increase in P and N uptake in shoots and with per cent root colonization in roots and spore number over the control ones.

Plants treated with dual inoculam of AM fungi and *azosprillium* showed higher P uptake when compared to control plants. Growth parameters such as plant height, dry weight of root and shoot, per cent root colonization (Fig C), spore number was high in AM fungi+ *Azosprillium* treated plants than that of AM fungi and *bacillus polymyxa* treated plants (Table 2). Tripartite inoculation of mycorrhizal fungi, *Azospirillium* and phosphate solubilising bacteria stimulated increase in plant growth parameters. Phosphorous and nitrogen content was high when compared to plants treated with dual inoculam. Increase in AM per cent of colonization was observed only after 90 days of mycorrhizal inoculation. The control plants failed to show significant growth due to the absence of AM fungal colonization, phosphate solubilising bacteria and nitrogen fixing organisms.

The effect of tripartite association of mycorrhizal fungi, nitrogen fixing bacteria and phosphate solubilising bacteria on growth and uptake of 'P' and 'N' has been a subject of interest in recent days. It is well known that the magnitude of plant response to any microbial inoculation is greatly affected by the Phosphorous (P) and nitrogen (N) content of the soil [16]. In particular P deficiency has been described as a main factor in restricting not only plant development but other biological process such as nitrogen fixation, owing to high requirement of P deficiency has been described as a main factor in restricting not only plant development but other biological processes such as biological nitrogen fixation, owing to the high requirement of P (as ATP) for the nitrogen fixation process [6]. The analysis that phosphorous is present in less available forms could be a factor limiting the bacterial survival. In this context, the role of AM fungi as phosphorous suppliers to the plant appears to be of great relevance. Thus in finger millet inoculation with AM fungi, *Azospirillium brasilense* and *Bacillus polymyxa* produced highest effect on either plant growth or nutrient uptake, together with a noticeable increase in mycorrhizal root colonization. The positive effect of *Azospirillum* inoculation is mainly attributed to improved root development and subsequent increase in the rate of water and mineral uptake.

Root colonization by AM fungi has been studied to increase absorption of minerals, particularly immobile nutrients, from soil by the host. Likewise, *Azospirillum*, one of the beneficial bacterial strains are known to play a pivotal role in fixing nitrogen in the roots by harnessing the atmospheric nitrogen and also have been reported to improve the fertility of soil. Beneficial bacterial flora can directly influence the physiology of the plants and in addition to interacting directly to beneficially influence the mycorrhizal relationship and/or plant growth [10], specific bacteria together with AM fungi have been studied to create a more indirect synergism that supports plant growth including nutrient acquisition [1] inhibition of plant pathogenic fungi, and enhancement of root branching [4]. In addition, most plant roots are colonized by mycorrhizal fungi and their presence also generally stimulates plant growth. However, most studies have reported the beneficial traits of root-colonizing bacteria and fungi separately and very few reports have demonstrated the synergistic effects of bacteria and AM fungi with respect to their combined beneficial impacts on plants. Beneficial effects of AM fungal inoculation in terms of fruit production were more pronounced in combined inoculation.

Table 2: Showing the effect of AM Fungi, *Azospirillium brasilense* , PSB (*Bacillus polymyxa)* on the growth response of *Eleusine coracana* (Finger millet).

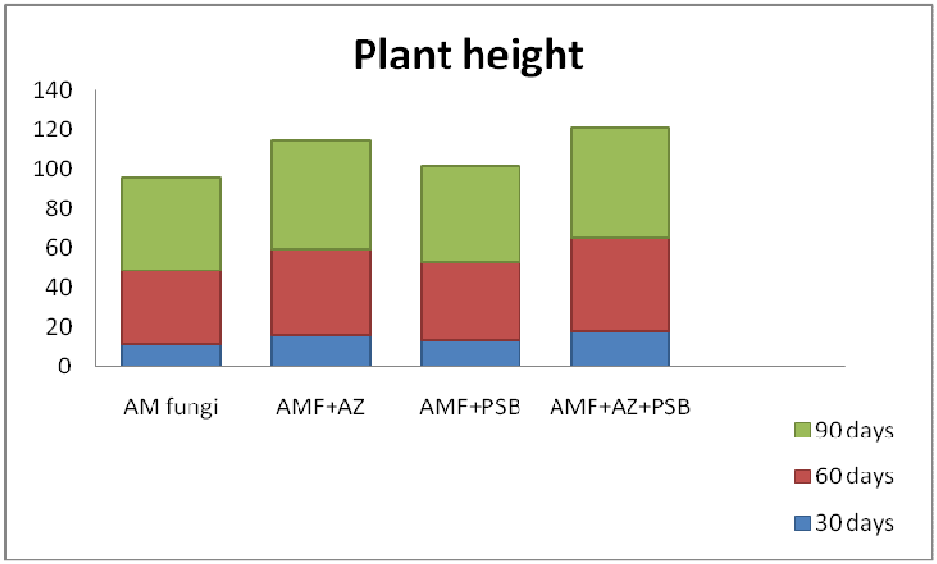
Treatments	Plant height	DWS	DWR	% root colonization	Spore number	P uptake	N uptake
30 days							
Control	4.63a	0.29a	0.24a	0.00a	0.00a	1.61a	3.11a
AM fungi	10.90b	0.63b	0.37b	51.1b	66.00b	2.11b	4.09b
AMF+AZ	16.06d	0.81cd	0.40d	54.7bcd	70.00c	2.60c	5.24cd
AMF+PSB	13.00bc	0.72bc	0.37c	52.06bc	74.00d	2.80cd	5.03c
AMF+AZ+PSB	18.00d	0.92d	0.50e	59.06e	87.00e	3.00d	5.40de
60 days							
Control	10.00a	0.61a	0.27a	0.00a	0.00a	4.9a	7.63a
AM fungi	37.73b	1.62b	0.64b	63.2b	74.00b	5.69b	9.23b
AMF+AZ	43.00cd	1.81bc	0.71d	70.6c	79.00c	6.03c	9.89bc
AMF+PSB	40.00bc	1.70bc	0.68c	68.0bc	82.00d	6.30d	9.73c
AMF+AZ+PSB	47.00d	1.89c	0.78e	70.1c	86.00e	6.56e	10.22e
90 days							
Control	16.53a	0.68a	0.40a	0.00a	0.00a	4.86a	9.36a
AM fungi	47.06b	2.18b	0.96b	67.2b	78.00b	5.96b	11.15b
AMF+AZ	55.36d	2.30c	1.10bcd	70.6bcd	95.00c	6.25c	12.23cd
AMF+PSB	49.03bc	1.80b	1.01bc	69.0bc	102.00d	6.55d	11.6bc
AMF+AZ+PSB	56.33d	2.79d	1.46d	75.2d	126.00e	6.77	12.40d

AZ- Azospirillium brasilense; PSB- phosphate solubalizing bacteria (Bacillus polymyxa); DWS- dry weight of shoot; DWR- Dry weight of root; P- Phosphorous; N- Nitrogen

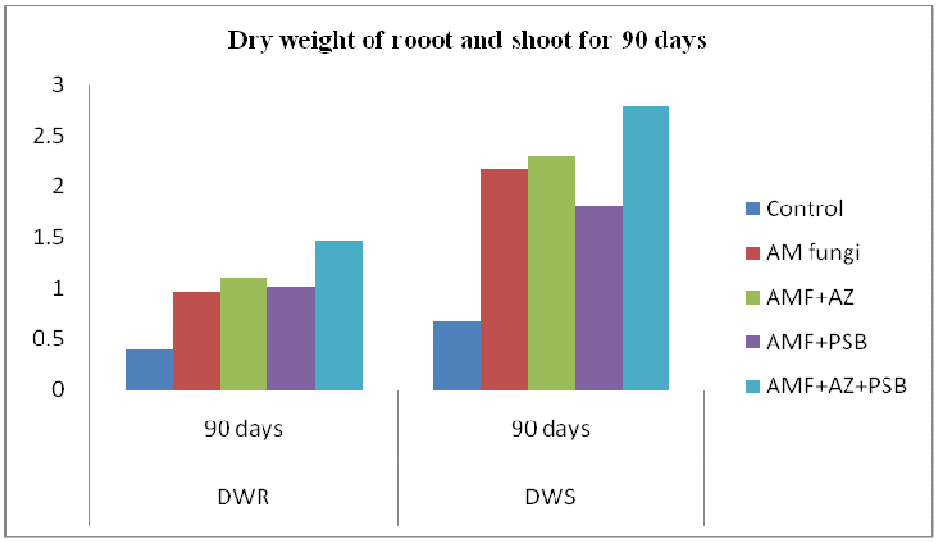
Means sharing a letter in columns are not significantly different according to Duncan's test P <0.05.

Fig: A, B, C showing plant height, dry weight of root and shoot and Per cent root colonization in *Eleusine coracana* (Finger millet).

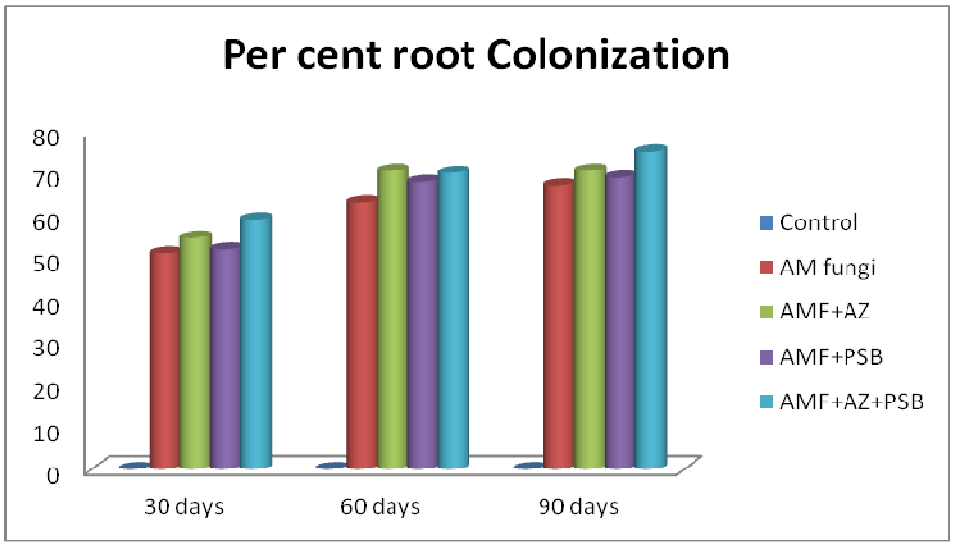
A)



B)



C)



CONCLUSION

In the present study, the co-inoculation of AM fungi, PSB and *Azospirillum* have probably acted similarly bringing about an increase growth and yield in finger millet. It is inferred that under appropriate management, the use of more efficient bio fertilizers have lead to an increased growth and biomass of finger millet. The present study have clearly shown that the combined application of bioinoculants like *Azospirillum* + *Azotobacter* + AM fungi played a significant role in improving the growth response and nutrient uptake of finger millet seedlings thereby producing good quality planting stock. These seedlings may perform better growth, survival and more biomass production in nutrient impoverished soil.

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