Effect of co-inoculation of AM fungi on growth, P uptake, acid and alkaline phosphatase activity in *Capsicum annum* L. Var. Pusa jwala.

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Abstract

Greenhouse pot experiments were carried out to Know the effect of co-inoculation of AM Fungi on plant growth, acid and alkaline phosphatase activity in *Capsicum annum* L. Var. Pusa jwala. AM Fungal inoculated plants showed a significant increase in plant height, biomass, fruit yield and Phosphatase activity. The better improvement was observed by the inoculation of *Rhizophagus fasciculatus* with *Glomus macrocarpum* combination as compare to single strain and control one. However, the best improvement was recorded with the three AM Fungal inoculants with increased in biomass, P uptake and fruit yield of *Capsicum annum* L. Var. Pusa jwala.

Keywords: Arbuscular mycorrhizal (AM) fungi, *Rhizophagus fasciculatus*, *Glomus macrocarpum,* acid and alkaline phosphatase activity, *Capsicum annum* L. Var. Pusa jwala.

Introduction

The fundamental problem, which the world faces today, is the rapidly increasing pressure of population on the appallingly limited resources of the land. To meet the ever increasing demand of expanding population, agriculture production has been raised through the abundant use of inorganic fertilizers, adopting multicropping system and liberal application of chemical pesticides fungicides, bactericides etc., Though the use of chemical fertilizers has increased the yield dramatically, it has also resulted in rapid deterioration of land and water resources apart from wastage of scarce resource.

The soil micro biota include species responsible for nutrient mineralization and cycling antagonists, species that produce substance capable of modifying plant growth and species that form mutually beneficial (symbiotic) relationships with plant roots. The importance of other soil micro organisms for plant growth is well demonstrated by many workers in recent day (Harely and Smith, 1997; Bagyaraj 2006; Lakshman, 2009, 2012). Arbuscular mycorrhizal (AM) fungi are associated with more than 90% of terrestrial plant families (Trappe, 1987). This fungi have amply demonstrated, their influence on the physiological benefits conferred by them potential host plants (Lakshman, 1996; Harely and Smith, 1997; Gupta *et al*., 2000). When, a plant becomes arbuscular mycorrhization there are significant changes in its physiology. The increase in stomata behaviour and photosynthesis of host plants along with increase in chlorophyll concentration due to inoculation of the AM fungi in different plant species as well. The importance of AM fungi for plant has now been fully appreciated. Inocula consisting of single species of a AM fungus are being tried to ensure a better performance of the crops. In

turn, this should lead to a more widespread ecological crop production with greater agricultural output and better profit in marginal soils, encouraging a higher environmental awareness.

Benefits of mixed inocula have been highlighted in the recent years but due attention has been paid to explore their practical application (Koomen *et al*., 1987: Hepper et al., 1987; Sieverding, 1988; Kumar, 1990; Harbanset al., 1995). In present study different AM fungi viz., single, dual and triple inoculation experiments were carried out on *Capsicum annum* L. under greenhouse conditions to characterize in terms of their plant growth promotion abilities. However, in order to known the possible synergistic effect of the microbial consortium on plant biomass yield, phosphatase activity and P (Phosphorus) content in shoot of *Capsicum annum* L. Var. Pusa jwala was undertaken.

Material and Methods

Soil sample collection: To isolate the dominant AM fungi, composite rhizospheric soil samples were collected from the *Capsicum annum* L. growing places. This was done by digging out by soil digger with a small amount of soil close to the plant roots up to 05-30cm depth, samples were kept in sterilized polythene bags with labelling and stored in refrigerator 4^0 C for further processing.

Isolation of dominant AM spores from soil samples:

Isolation of indigenous AM fungal spores *G. macrocarpum*, *G. bagyarajii* and *R. Fasciculatus* was done by 'Wet sieving and decanting technique' following the procedure of (Gerdemann and Nicolson, 1963). For this, 50g of soil was soaked in 250ml water for 24 hr. the supernatant was then passed through a gradient of sieves with pore sizes ranging from 850µm to 45µm arranged one above the other in an ascending order. Each sieve was then washed in water which was filtered through Whatman No.1 filter paper. This filter paper was then observed under a stereo binocular microscope to observe various kinds of spores; it was mounted on Polyvinyl lactic acid (PVLA) for further studies.

Quantification of AM spores and identification of AM fungi:

This was done by the Grid line intersect method (Giovannetti and Mosse, 1980; Adholeya and Gaur, 1994). Spores were counted under a stereobionocular microscope with a counter. The AM spores (*G. macrocarpum*, *G. bagyarajii* and *R. Fasciculatus*) were identified with the identification manuals written by (Walker, 1983; Schenck and Perez, 1990; and Mukerji, 1996).

Mycorrhizal root colonization:

Root samples were washed and put in 10% KOH autoclaved for 2hr. and neutralized in 2% HCL and washed in distilled water and stained in 0.05% cotton blue in lactophenol and the root colonization was done by the rapid clearing and staining method of (Philips and Hayman, 1970). The percentage of AM root colonization was calculated by using the following formula...

$$\text{Per cent of root colonization (\%)} = \frac{\text{No of root bits colonization}}{\text{Total number of root bits observed}} \times 100$$

Mass production of AM spores:

Dominant AM spores of *R. fasciculatus, G. macrocarpum* and *G. bagyarajii* were isolated from rhizospheric soil of *Capsicum annum* L. growing field at Haveri in Karnataka and the study location geographical area is located in between 15° 30' and 15°50' north latitude and 75° 07'

and 75° 38' east longitude. The geographical area is 485156 Hectares. The dominant spores were mass multiplied with *Sorghum vulgare* L. as host plant.

Pot mixture preparation:

The soil was sterilized for two consecutive days at 15 lb pressure with 121^0 C temperatures, and for two hours. Earthen pots measuring (25×30 cm) (length× breadth) were selected, each pot was filled with 4kg of sterile garden soil: pure sand in (1:1 v/v) ratio. To each pot 20g dry mixed inoculum of AM fungi of different species in combination were placed just 4cm below the soil surface of each experimental pots. And the different treatments used during the present investigation were adopted as follows...

1. Control (Non inoculated)
2. *R. fasciculatus* (Thaxt.) C. Walker & A. Schüßler
3. *G. macrocarpum* Tul. & Tul.
4. *G. bagyarajii* V.S. Mehrotra
5. *G. macrocarpum + R. fasciculatus*
6. *G. bagyarajii + R. fasciculatus*
7. *G. macrocarpum+ G. bagyarajii*
8. *G. macrocarpum+ G. bagyarajii + R. fasciculatus*

The mycorrhizae inoculated plants were watered whenever, it was necessary to the experimental pots, where the *Capsicum annum* L. Var. Pusa jwala were maintained. Hoagland's nutrient solution without phosphorus (2ml/pot) was added to each plant after regular intervals of 15 days. Each treatment was replicated three times. All the plant parameters were measured and recorded after 90 days of microbial inoculation.

Analysis of growth parameters:

Plants were harvested after 90 days to understand the effect of different AM Fungal inoculation on growth parameters such; as Fresh and dry weight of shoots and roots. For dry weight Plants were oven dried constantly for 2 hours at 70^0 C. Estimation of phosphorus was done by 'Vanadomolybdo phosphoric yellow colour method' (Jackson, 1973). The acid and alkaline phosphatase activity was estimated by using the standard protocol of by (Plummer, 1987).

Statistical analysis:

All results were analysed using analysis of variance, (ANOVA), followed by post hoc test through computer software SPSS 16.0 version. Means were ranked at $P \leq 0.05$ level of significance using Duncan's Multiple Range Test of comparison.

RESULTS AND DISCUSSION

The results of the different treatments of AM Fungi inoculation (single, dual and triple) have been presented in (Table 1). In present investigation, triple inoculation of AM Fungi is significantly favour in increased all the growth parameters and fruit yield of *Capsicum annum* L. Var. Pusa jwala. There was an increase in P uptake in shoots and per cent root colonization in roots and spore number over the (control) non inoculated plants.

Single species inoculation of *R. fasciculatus* influenced on plant growth and biomass yield in *Capsicum annum* L. compare to other species *G. macrocarpum* and *G. bagyarajii*. However, the dual inoculation of *G. macrocarpum* and *R. fasciculatus* showed a favourably increased plant height and biomass yield, P uptake in shoots and acid alkaline phosphatase activity of rhizosphere soils over the (control) non inoculated plants. However, the triple inoculation has brought the most significant growth in all parameters as compared to control single or dual inoculation, it shows that more diversity leads to the significant increase in all considerations.

Growth parameters such as, Shoot length, Per cent root colonization, P uptake shown in (Figs A, B, C), spore number was high in triple inoculation and followed by dual inoculation of *G. macrocarpum* Tul. & Tul + *R. fasciculatus* (Thaxt.) C. Walker & A. Schüßler., and single inoculation of *R. fasciculatus* (Thaxt.) C. Walker & A. Schüßler. 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 microorganisms.

The effect of combined inoculation of mycorrhizal fungi on growth and P uptake 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) content of the soil (Paula *et al*., 1992). In particular, 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 (Giller *et al*., 1995). The analysis that phosphorus 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 (sieverding, 1995; Bagyaraj, 2006; Lakshman, 2009, 2013). Thus, in *Capsicum annum* L. Var. Pusa jwala. inoculation with different combination of AM fungi produced highest effect on either plant growth or nutrient uptake, together with a noticeable increase in mycorrhizal root colonization. The positive effect of AM fungi inoculation is mainly attributed to improved root development and subsequent increase in the rate of water and mineral uptake. Our results are corroborates with the results of (Koomen et al. 1987; Gurumurthy and Srinivasa, 1996; Sieverding, 1998; Lakshman, 2012).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 fungi. The hyphae of the AM fungi cause the aggregation of the soil particles (Linderman, 1988; Lakshman, 1992; Filion *et al*., 1999). There is an increase in the rate of movement of water and nitrates from the bulk soil to the root surface (George et al., 1995). Thus, the number of AM propagules and the percentage of root colonization increase. And very few reports have demonstrated the synergistic effects of AM fungi with respect to their combined beneficial impacts on plants (Trappe, 1987). Beneficial effects of AM fungal inoculation in terms of fruit production were more pronounced in combined inoculation.

Table 1. Showing the effect of co-inoculation to *Capsicum annum* L. Var Pusa Jwala on growth response, P content, acid and alkaline phosphatise activity at 90 days.

G.ma- G. macrocarpum, *G. ba- G. bagyarajii*, *R. Fa- R. fasciculatus*

SL-Shoot length(cm), FWS-Fresh weight of shoot(g), DWS-Dry weight of shoot(g), PRC-Per cent root colonization, SN- Spore number/ 50 g soil,

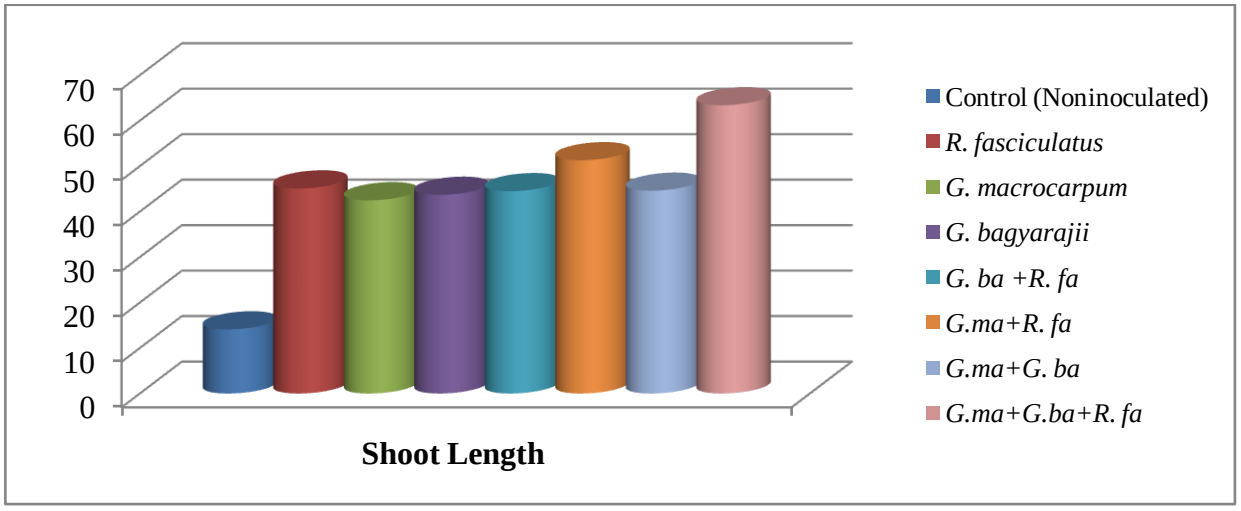
TREATMENT	SL	FWS	DWS	FWR	DWR	PRC	SN	NFr	P% in shoot	ACP µg/g soil	ALP µg/g soil
Control (Noninoculated)	14.1±1.3c	3.4±0.09a	1.8±0.2e	0.49±0.1c	0.19±0.2b	34.1±1.2b	24.2±0.0b	1.9±0.02a	0.05±0.0e	52.0±0.0c	39.2±0.0e
R. fasciculatus	45.1±2.2e	5.5±0.7c	1.9±0.1a	0.66±0.2a	0.13±0.0a	57.3±0.0b	187±0.0d	2.3±0.03c	0.11±0.0b	61.0±0.0a	46.1±0.0d
G. macrocarpum	42.5±3.0b	5.1±1.0b	1.4±0.02b	0.82±0.2c	0.32±0.0a	49.0±0.0c	181±0.0a	2.9±0.2e	0.09±0.1c	65.1±1.1b	44.2±0.0a
G. bagyarajii	43.7±2.3a	5.3±1.1d	1.5±0.1d	0.91±0.0b	0.44±0.0c	47.0±0.0d	178±0.5b	2.4±0.5a	0.09±0.0a	64.2±2.0a	44.1±0.0d
G. ba +R. fa	44.5±2.1d	8.4±1.4d	3.1±0.5c	1.0±0.0c	0.52±0.0d	56.1±0.0b	189.1±0.0a	3.1±0.9b	0.13±0.0e	63.3±1.1e	47.2±0.0a
G.ma+R. fa	51.3±5.0b	11.3±2.0b	5.4±0.9a	1.5±0.0b	0.73±0.0c	61.2±0.0a	193.0±0.0d	9.8±1.0c	0.17±0.0d	76.2±2.0a	53.1±0.0c
G.ma+G. ba	44.6±5.2e	7.5±1.7c	2.7±0.4a	1.3±0.0d	0.67±0.0b	52.1±0.0a	152.2±0.0c	8.4±1.1d	0.12±0.0a	66.1±1.5d	51.3±0.0e
G.ma+G.ba+R. fa	63.4±3.1a	14.3±2.1e	8.7±1.0b	2.03±0.9c	0.91±0.0d	78.1±0.0d	204.0±0.0b	13.2±1.9e	0.23±0.0c	78.2±1.0e	64.2±0.0b

P%- Phosphorus content in shoot, ACP-Acid Phosphatase, ALP-Alkaline Phosphatase, NFr- Noumber of fruits.

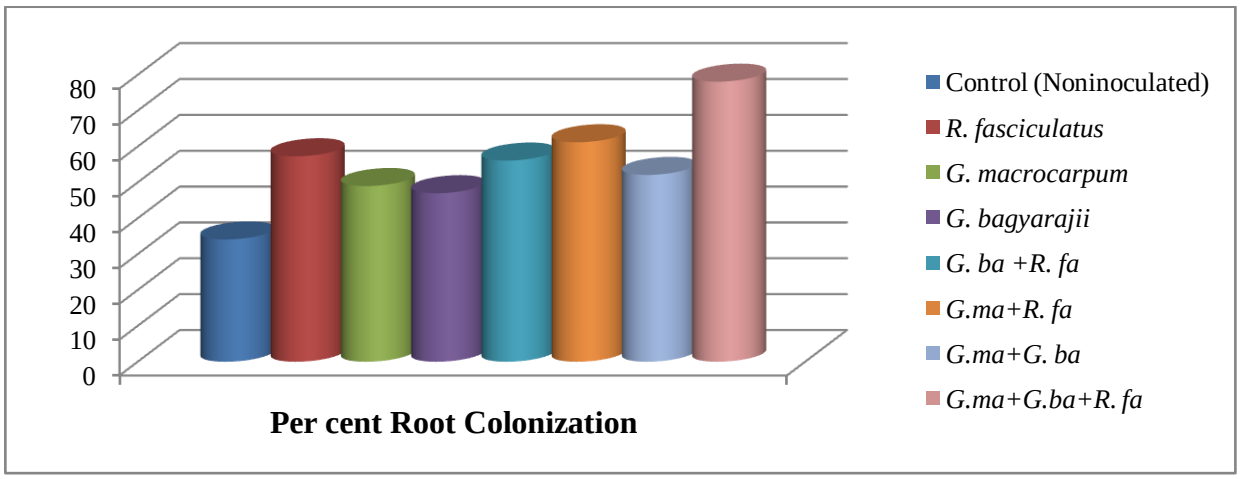
Means sharing letter in the column do not differ significantly at P=0.05 level by DMRT.

Fig A, B, C Showing the effect of co-inoculation to *Capsicum annum* L. Var Pusa Jwala on shoot length, per cent root colonization, P uptake at 90 days.

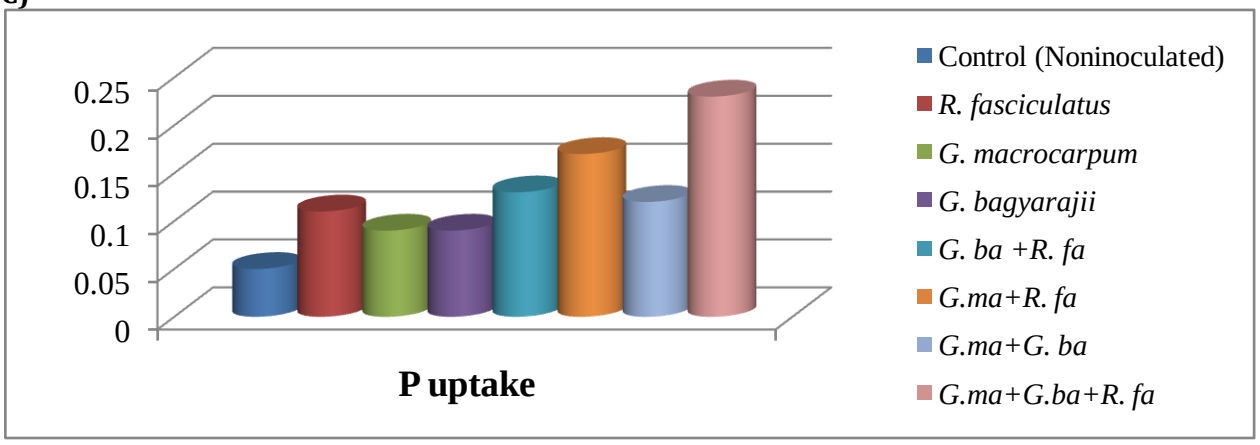
A)



B)



C)



G.ma- G. macrocarpum, G. ba- G. bagyarajii, R. Fa- R. fasciculatus

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