Effect of different AM Fungi inoculation on growth, biomass, yield enhancement and nutrient uptake in cultivar Sankeshwar of Capsicum annuum L

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***Abstract*— The selection of AM fungi is required to characterize the native AM fungi population from the soil types. The recent studies have clearly reported that these fungi are host preference in enhancing the growth, yield and nutrient. In the present study six indigenous AM fungi were selected from chillies growing fields of Haveri. These six AM fungi were inoculated to cultivar Sankeshwar of Capsicum annuum L. at green house conditions. Plants harvested at 60 and 90 days after inoculation. The results obtained from the experiments were clearly evidence that the positive influence of Glomus macrocarpum Tulasne & Tulasne. on cultivar Sankeshwar of Capsicum annuum L. plants in increasing plant height, root length, fresh weight of shoot and fresh weight of root, % root colonization, spore number and P uptake in shoot and root. Similarly the improvement of number of fruits was higher. The second AM fungus Rhizophagus fasciculatus (Thaxt.) Walker & Schüßler was influenced in all the different parameters it is followed by Glomus mosseae respectively. And thus an indigenous AM fungi play an important role over the control plants or non inoculated plants. This is mainly due to AM fungal species differ considerably in their efficiency to colonize and influenced plant growth biomass yield and nutrient uptake.**

***Index Terms*— Indigenous AM fungi, Capsicum annuum L., Glomus macrocarpum, Rhizophagus fasciculatus, Glomus mosseae, per cent root colonization, plant biomass, spore number, Haveri.**

I. INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are obligate biotrophic organisms belongs to Glomeromycota that live symbiotically with the roots of plants. The nutrient deficient soils usually harbor more AMF [1]. Hence, their ecological significance is enormous. AMF play an important role in plant nutrition and soil nutrition [2], [3]. Nearly 90% of land plant species harbor AMF. AMF associations were also found in aquatic habitats [4]. AMF help in plant nutrition and disease resistance and also provide an alternative to chemical fertilizers particularly in land reclamation, habitat restoration and sustainable agriculture. Microorganisms have been found to solubilize the low soluble calcium phosphate via the production of exo-metabolites (Phosphatase) and make them available to plants [5]. Alkaline Phosphatase (ALP) has long been suspected of being involved in phosphate efflux from arbuscules because of strong ALP activities at arbuscules [6], [7]. Arbuscular mycorrhizal fungi (AMF) are important in mobilizing phosphorus (P) nutrition in many soils through hyphal transport to the plant [8]. Nutrition exchange between the two symbionts is at the core of the arbuscular mycorrhizal (AM) association and reciprocal transfer is a requirement for a functioning symbiosis [9]. The first model to quantify the contribution of the external fungal mycelium to plant P uptake was developed by [10]. The current day emphasis is on sustainable agriculture, which uses less of chemical inputs like fertilizers and pesticides having adverse effect on soil health, fertility and environment. Thus use of microbial inoculants play an important role in sustainable agriculture [11], [12]. Arbuscular mycorrhizal fungi are known to improve the nutritional status, growth and development of plants, protect plants against root pathogens and offer resistance to drought and salinity [13]. Though these fungi are not host specific, recent studies have clearly brought out host preference in arbuscular mycorrhizal fungi. Host preference has been reported in many plants like *Casuarina euisetifolia* [14]; *Tectona grandis* [15]; *Garcinia indica* [16], and a few medicinal plant species like *Phyllanthus amarus* and *Withania somnifera* [17] and *Coleus forskohlii* [18].

Capsicum annuum L. is one of the important pepper plants widely cultivated all over the world. It is the most popular condiment being used to add zest and flavor, a large green, non-pungent forms, and eaten row salads. It yields "paprika" which is used as flavoring and coloring material in cookery. Commercially large quantities of paprika are used in the manufacture of sausages and other meat products. Cultivar Sankeshwar of *Capsicum annuum* L. is an important agricultural crop, not only because of its economic importance, but also due to nutritional and medicinal value of its fruits. *Capsicum annuum* L. are the excellent source of natural colors and antioxidant compounds [19]. A wide spectrum of antioxidant vitamins, carotenoids, capsaicinoids and phenolic compounds are present in hot pepper fruits. The intake of these compounds in food is an important health-protecting factor by prevention of widespread human diseases. As consumption continues to increase, hot peppers could provide important amounts of nutritional antioxidants to the human diet. Screening of efficient AM fungi for this plant was not investigated. Therefore, the purpose of this study was to select a suitable AM strain for its improvement of growth biomass yield and Phosphorus uptake in mycorrhizae inoculated and non inoculated plants.

II. MATERIALS AND METHODS:

Collection, Surface Sterilization of Seeds and preparation of seedlings:

Cultivar Sankeshwar of *Capsicum annuum* L. was procured from Horticulture Research Station, Haveri (Devihosur) – 581110 (University of Horticultural Sciences, Bagalkot India). The study area of its geographical location lying in

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between 15° 30' and 15°50' north latitude and 75° 07' and 75° 38' east longitude. Cultivar Sankeshwar of *Capsicum annuum* L. seeds were thoroughly washed under running water and then with distilled water. *Capsicum* seeds were placed in 0.4% mercuric chloride ($HgCl_2$) solution for 1-2 minutes then washed for 3-4 times in sterile distilled water to ensure the surface sterilization and complete removal of $HgCl_2$ before sowing the seeds. Surface sterilized seeds were placed in measuring 25 × 50 cm (Length× Breadth) diameter broad pots having 8 kg sterilized soil containing sand: soil (1:1/v:v), to get seedlings about 10 to 15 cm in height, equal height seedlings were selected and they were transplanted in to the experimental pots.

AM fungal Inoculum, Recovery and Estimation of Mycorrhizal Spores:

The AM fungal spores were recovered from the rhizospheric soils collected from different areas where the cultivar Sankeshwar of *Capsicum annuum* L. is growing in Haveri district of North Karnataka by adapting wet sieving and decanting technique [20]. AM fungal spore number in the soil suspension was determined by using the procedure as described by [21]. The most dominant species such as *Acaulospora leavis*, *Gigaspora margarita*, *Glomus macrocarpum*, *Glomus mossae*, *Rhizophagus fasciculatus*, and *Sclerocystis dussii*, were selected for the experiments. Then these six AM fungal species were mass multiplied by using most suitable host *Sorghum vulgare* Pers., using sterilized soil and sand mixture (3:1 v/v), under greenhouse conditions.

Experimental Design:

The soil used in the study has pH–7.5±0, EC–0.32 dS-1/m, organic carbon–0.45%, and available macronutrients like N–182 kg/ha, P_2O_5– 49.70 kg/ha, K_2O–180 kg/ha with micronutrients like Ca–18.00 mg/100g soil, Mg–7.8 mg/100g soil, Fe– 6.57 mg/kg, Zn–6.40 mg/kg, Mn– 5.15 mg/kg, Cu– 0.10 mg/kg. The mixture of mycorrhizae colonized *Sorghum* root bits and soil containing mycelia and spores (158-189 spores/10 g soil) was served as inoculum. Experimental pots measuring 25 × 30 cm (Length× Breadth) diameter having 4 kg sterilized soil were watered on every alternate day and provided Hoagland's nutrient solution without P to each experimental pot at every fortnight. Plants were maintained in greenhouse at Department of Botany, Karnatak University Dharwad. The treatments were as follows.

T1: Soil without fungal inoculum (Control or Non – Mycorrhizal)

T2: Soil with AM Fungus *Acaulospora laevis* Gerd. & Trappe.

T3: Soil with AM Fungus *Gigaspora margarita* Becker and Hall.

T4: Soil with AM Fungus *Glomus macrocarpum* Tulasne & Tulasne.

T5: Soil with AM Fungus *Glomus mosseae* (Nicolson &Gerdemann) Gerdemann & Trappe.

T6: Soil with AM Fungus *Rhizophagus fasciculatus* (Thaxt.) Walker & Schüßler.

T7: Soil with AM Fungus *Sclerocystis dussii* (Pat.) Höhn.

Harvest and Analysis of Growth parameters:

At 60 and 90 days after transplanting (DAT), plants were harvested to record the total plant biomass (dry weight of shoot and root) and plant height. Dry weight of root and shoot was determined after constant drying at 70^0 C for 48 hrs under hot air oven. All the growth parameters were measured in triplicate. Phosphorus content in shoot was determined calorimetrically by the vanadomolybdate/ phosphoric-yellow colour method outlined by [22]. Nitrogen content of shoot was determined by Microkjeldahl method [22]. For the fruit analysis we harvest the Fruits at 90 days after sowing.

Root Colonization: The per cent root colonization was evaluated microscopically followed by clearing of roots in 10% KOH and staining with 0.05% trypan blue in lactophenol according to method described by [23]. The following formula was used to calculate the root colonization according to [24].

$$\text{Per cent mycorrhizal colonization (PMC)} = \frac{\text{Number of root bits colonized}}{\text{Number of total segments examined}} \times 100$$

Statistical analysis: All the data were analyzed according to analysis of variance (ANOVA) by using the SPSS-16 software. Within each variable, significant difference among the means were assessed with the Duncan's multiple Range Test (DMRT) (P=0.05).

III. RESULTS:

In the present study, Six different indigenous AM fungal species namely, *Acaulospora leavis*, *Gigaspora margarita*, *Glomus macrocarpum*, *Glomus mossae*, *Rhizophagus fasciculatus*, and *Sclerocystis dussii*, were used to screen the efficient AM fungus to improve the plant growth, biomass production and fruit yield of cultivar Sankeshwar of *Capsicum annuum* L. under greenhouse conditions. Results revealed that, the mycorrhizal inoculation influenced significantly increased plant growth, biomass production and fruit yield in cultivar Sankeshwar of *Capsicum annuum* L. compared to non-mycorrhizal plants. It was also noted that, the rate of increased plant growth was varied with each AM fungal species (Fig - 2).

Cultivar Sankeshwar of *Capsicum annuum* L. had shown improved plant growth parameters inoculated with six different AM fungi compared to non-mycorrhizal (control) plants. Increased plant growth and biomass production was recorded at both stages of harvest i.e. 60 DAT and 90 DAT. Maximum influence of AM fungal inoculation was observed at 90 DAT compared to 60 DAT. Significantly increased plant height i.e. shoot length (74.23 cm) and root length (34.33cm), biomass production i.e., shoot dry weight (6.89 g/plant) and root dry weight (3.26 g/plant) was observed with AM fungus *Glomus macrocarpum* inoculation compared to other five AM fungi inoculated and control plants as shown in Table 1 and Fig-1. Results also revealed that, the second best AM fungus for cultivar Sankeshwar of *Capsicum annuum* L. was *Rhizophagus fasciculatus*. Least increased plant heights was observed with AM fungus *Gigaspora margarita* and *Sclerocystis dussi* inoculation, but it was significant over the non-mycorrhizal plants. Greatly improved P-uptake was observed in *Capsicum annuum* L. Var. Sankeshwar shoot inoculated with AM fungus *Glomus macrocarpum* compared to other AM fungi inoculated and non-mycorrhizal ones. Fruit yield was recorded and it was significant with mycorrhizal inoculation compared to non-mycorrhizal ones as shown Table 1.

Similarly, increased mycorrhizal status in cultivar

Sankeshwar of *Capsicum annuum* L. root and rhizosphere was observed at all stage of harvest. It was found that, maximum per cent mycorrhizal colonization (PMC) (93.67 %) in root and more mycorrhizal spore number (MSN) (302.00 /50g soil) in the rhizosphere with AM fungus *Glomus macrocarpum* inoculation compared to other AM fungi inoculated plants and non-mycorrhizal ones. Overall the second best AM fungus to improve cultivar Sankeshwar of *Capsicum annuum* L. growth and fruit yield was *Rhizophagus fasciculatus* among the AM fungal species used for inoculation and the inoculations of different AM fungi with respect to harvest period, growth parameters and nutrient uptake are significantly (at 0.01 and 0.05 level) correlate with each other shown in (Table-2).

Table 1: Showing the influence of different AM fungi inoculation on growth, yield, P and N uptake in cultivar Sankeshwar of *Capsicum annuum* L. at two intervals (i.e. 60 and 90 DAT).

Treatments	Shoot			Root			Yield	Mycorrhizal Status		Nutrient Uptake	
	Length (cm)	Fresh Weight (g)	Dry Weight (g)	Length (cm)	Fresh Weight (g)	Dry Weight (g)	Number of Fruit/ Plant	PMC	MSN	P (%)	N (%)
60 DAYS											
[a]NM	34.63±0.18a	09.66±0.32a	1.64±0.04a	11.60±0.15a	1.75±0.09a	0.38±0.00a	1.96±0.03a	[b]NR	[b]NR	0.08±0.00a	0.99±0.06a
A. laevis	42.37±0.07d	12.57±0.24d	3.03±0.04cd	18.30±0.10d	2.47±0.03cd	0.70±0.00d	3.57±0.31b	59.33±0.90b	187.00±0.58c	0.16±0.01c	1.43±0.09b
G. margarita	38.70±0.35b	10.68±0.16b	2.46±0.07b	14.53±0.03b	2.10±0.06b	0.49±0.00b	2.30±0.10a	55.67±0.88a	169.00±0.00a	0.12±0.0b	1.33±0.09b
G. macrocarpum	51.63±0.32g	15.73±0.12g	4.30±0.15f	24.87±0.09g	3.77±0.08f	1.30±0.07f	6.03±0.15e	75.33±0.88e	204.50±0.00f	0.21±0.0e	2.28±0.06d
G. mossae	45.30±0.06e	13.97±0.07e	3.23±0.12d	18.63±0.12e	2.60±0.06d	0.77±0.03d	4.17±0.17c	64.33±0.91c	194.33±0.09d	0.18±0.01d	1.85±0.03c
R. fasciculatus	47.00±0.06f	14.73±0.12f	3.70±0.00e	19.80±0.00f	2.89±0.01e	0.99±0.01e	4.80±0.00d	68.00±0.57d	199.30±0.00e	0.19±0.01de	2.02±0.04c
S. dussii	40.27±0.09c	11.90±0.00c	2.87±0.03c	15.30±0.15c	2.27±0.09bc	0.59±0.00c	3.20±0.00b	57.67±0.66ab	181.33±0.88b	0.14±0.01bc	1.10±0.06a
90 DAYS											
[a]NM	45.83±027a	10.36±0.12a	2.70±0.15a	16.63±0.22a	2.09±0.06a	0.80±0.00a	4.96±0.03a	[b]NR	[b]NR	0.09±0.00a	1.18±0.03a
A. laevis	53.86±0.03d	13.26±0.13c	3.90±0.00c	22.90±0.06d	5.20±0.10d	2.36±0.09d	9.50±0.29d	79.33±0.67b	246.66±0.00c	0.14±0.02b	1.64±0.12b
G. margarita	49.90±0.06c	12.16±0.21b	3.43±0.23b	17.43±0.18b	4.10±0.06b	1.83±0.03b	5.86±0.03b	70.33±0.67a	213.00±1.33a	0.14±0.00b	1.46±0.07ab
G. macrocarpum	74.23±0.17g	17.46±0.20f	6.89±0.05f	34.33±0.33f	7.93±0.09g	3.26±0.18f	13.63±0.37f	94.66±2.40d	306.66±1.00e	0.25±0.00e	3.10±0.16d
G. mossae	58.30±0.35e	14.90±0.00d	4.40±0.20d	23.30±0.30d	6.40±0.06e	2.60±0.05de	10.33±0.34d	86.67±1.33c	293.67±3.84d	0.19±0.01cd	2.30±0.16c
R. fasciculatus	67.56±0.34f	16.20±0.00e	5.66±0.12e	26.73±0.27e	6.92±0.04f	2.80±0.00e	12.16±0.44e	93.67±0.66d	302.00±1.20e	0.21±0.01d	2.28±0.07c
S. dussii	48.10±0.10b	12.46±0.20b	4.20±0.06cd	19.56±0.19c	4.70±0.10c	2.10±0.00c	7.83±0.03c	77.76±0.19b	230.66±1.00b	0.17±0.01c	1.27±0.06a
F-Statistics Values											
Days (D)	15240.23**	149.79**	537.71**	3036.46**	5211.46**	2144.83**	582.97**	1163.60**	9129.35**	17.5**	46.44**
Treatment (T)	2883.81**	394.87**	191.14**	1590.15**	631.33**	156.31**	207.01**	1817.39**	9743.71**	79.79**	89.40**
D× T	330.28**	3.96*	15.11**	68.52**	178.86**	37.33**	6.36**	38.30**	454.53**	3.13*	3.86*

**, * significant at P=0.001, P=0.01 respectively. The values represents mean of triplicate. The same alphabet in the column represents no significance.
[a] – NM- Non Mycorrhizal, [b]- NR- Not Recorded, PMC- Per cent Mycorrhizal Colonization, MSN – Mycorrhizal Spore Number, P- Phosphorus, N- Nitrogen.

Table 2: Showing the Pearson's correlation coefficients (r) for plant growth, yield and nutrient status with inoculation of different AM Fungi in cultivar Sankeshwar of *Capsicum annuum* L

	TR	SL	SFW	SDW	RL	RFW	RDW	NF/P	PMC	MSN	P	N
DAYS	0	0.664**	0.241	0.546**	0.483**	0.718**	0.802**	0.555**	0.307*	0.360*	0.18	0.271
TR		0.256	0.465**	0.405**	0.259	0.303	0.266	0.297	0.601**	0.582**	.0531**	0.267
SL			0.847**	0.959**	0.951**	0.950**	0.922**	0.960**	0.700**	0.736**	0.778**	0.852**
SFW				0.895**	0.914**	0.773**	0.705**	0.913**	0.806**	0.801**	0.938**	0.932**
SDW					0.964**	0.914**	0.879**	0.948**	0.778**	0.794**	0.867**	0.860**
RL						0.885**	0.841**	0.972**	0.733**	0.745**	0.868**	0.910**
RFW							0.981**	0.902**	0.755**	0.798**	0.721**	0.768**
RDW								0.873**	0.722**	0.764**	0.654**	0.695**
NF/P									0.732**	0.745**	0.852**	0.907**
PMC										0.989**	0.816**	0.688**
MSN											0.804**	0.693**
P												0.869**

**, * Correlation is significant at the 0.01 and 0.05 level respectively (2- tailed).

Note: SL: Shoot length, SFW: Shoot Fresh weight, SDW: Shoot dry weight, RL: Root length, RFW: Root fresh weight, RDW: Root dry weight, NF/P: Number of fruit per plant, PMC: Per cent mycorrhizal colonization; MSN: Mycorrhizal spore number, N: Nitrogen, P: Phosphorus, TR- Treatments.

Fig.1: Showing the effect of inoculation of different AM fungi on Dry weight of shoot and root in cultivar Sankeshwar of *Capsicum annuum* L. at 90 days (DAT).

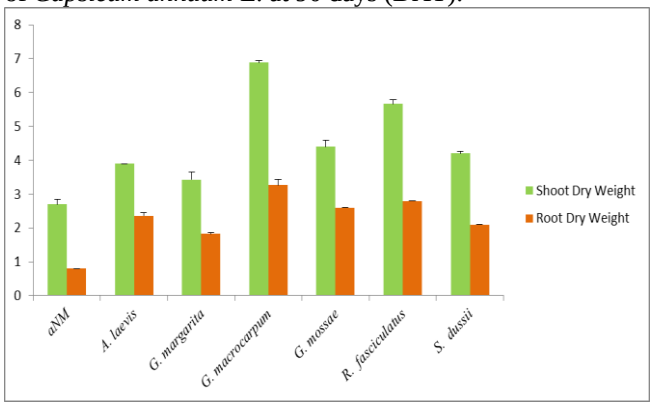
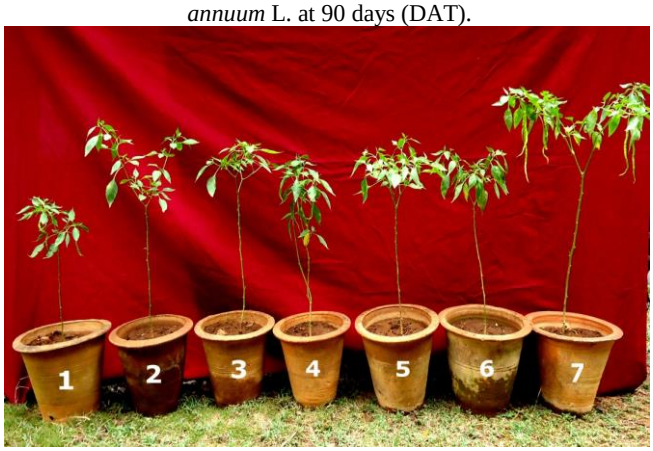


Fig.2: Showing the effect of inoculation of different AM fungi on height and number of fruits in cultivar Sankeshwar of *Capsicum annuum* L. at 90 days (DAT).



Note: 1-Control, 2-*Gigaspora margarita*, 3-*Acaulospora leavis*, 4-*Glomus mossae*, 5- *Rhizophagus fasciculatus*, 6-*Sclerocystis dussii*, 7- *Glomus macrocarpum*

IV. DISCUSSION:

Results obtained from the experiments gave the clear evidence that, the positive influence of mycorrhizal inoculation on cultivar Sankeshwar of *Capsicum annuum* L. growth responses. The dry weight of both shoot and root (biomass) was more in plants colonized with *Glomus macrocarpum* and the least with *Gigaspora margarita* and *Sclerocystis dussi*. The present experimental findings revealed that, the AM fungi play an important role in enhanced plant growth over the control plants. AM Fungi also provides platform for the growth and development of plants. Arbuscular mycorrhizal fungal species differ considerably in their efficiency to colonize and influence plant growth [25]. Host response also differs with fungal species and with geographic isolate within a species. The extent of response may also be due to changes in efficiency of different endophytes during the growing season [26], to varying uptake or exclusion capabilities of AM fungi for different element or a change in soil environment itself during the season [27].

Arbuscular mycorrhizal (AM) association with plants will increase uptake of mineral nutrients, especially phosphorus in conditions of low 'P' availability [28], [29] reduce susceptibility of plants to certain pathogens [30], increase seed production [31] and improve health and vigour of the seedlings [32], [33]. The increased plant growth and biomass accumulation of AM fungi inoculated plants strongly depends on their ability to access minerals from the soil. Therefore, positive effects of tested AM fungi on phosphorus content could be related to the ability of symbiotic fungi to enhance soil phosphorous depletion zones around roots [34], [35], [36]. According to earlier workers [37], [38] enhanced uptake of phosphorous is generally regarded as the most important benefit that, the AM fungi provides to their host plants and

plant phosphorous status is often the main controlling factor in the plant fungal relationship.

V. CONCLUSION:

It can be concluded from the experimental results that, cultivar Sankeshwar of *Capsicum annuum* L. inoculated with AM fungi have showed increased plant growth, biomass production and fruit yield. This indicated that, the mycorrhizal fungi offers maximum benefits to the experimental plants under varied conditions. Among the six AM fungal species, *Glomus macrocarpum* Tulasne & Tulasne. was found to be the most promising AM fungus to enhance the plant growth and yield of cultivar Sankeshwar and *Rhizophagus fasciculatus* (Thaxt.) Walker & Schüßler. was found to be second best AM fungus. Therefore, application of these kinds of soil inhabiting beneficial microorganisms will be more cost effective and eco-friendly to retain and to enhance the soil fertility and agricultural productivity.

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