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Research Article

INFLUENCE OF ARBUSCULAR MYCORRHIZAL FUNGI AND *AZOSPIRILLUM* CO-INOCULATION ON THE GROWTH CHARACTERISTICS, NUTRITIONAL CONTENT AND YIELD OF TOMATO CROPS GROWN IN SOUTH INDIA

[*]Viswanathan Guru[1], Panchaksharam Tholkappian[2], and Kurusangu Viswanathan[3]
[1,2]Department of Microbiology, Faculty of Science, Annamalai University, Annamalainagar -608002, Tamilnadu, India
[3]Institute of Animal Nutrition, Tamilnadu Veterinary and Animal Sciences University, Livestock Reserach station Campus, Kattupakkam- 603203, Tamilnadu, India
**Author for Correspondence*

ABSTRACT
To assess the influence of co-inoculation of AM fungi and *Azospirillum* on the growth characteristics, quality, nutrient contents and yield of tomato crop varieties, pot culture experiments were conducted during May to August 2009 at Chidambaram, Tamilnadu. The plant growth effects, number of fruits per plant, yield per plant, nutrient contents, available soil N and P, percent root colonization and the quality of fruits were measured for the singly inoculated, co-inoculated and un-inoculated control crops. Overall, the crops that were co-inoculated with the bio-inoculants expressed good growth, nutritional characteristics,better yield and quality fruits.

Key Words: *AM fungi; Azospirillum; Organic fertilizers; Tomato*

INTRODUCTION
Tomato (*Lycopersicon esculentum Mill.)* is one of the widely grown and consumed vegetable crops in the world. India's annual produce of tomato accounts for nearly, 7.1 million tones and among the states, Tamil Nadu stands seventh in tomato production. The production of tomato is greatly influenced by multiple factors including but not limited to the cultivars, type of soil, fertilizers used. The nutrients such as N, P, K, Mg and Ca are required by tomato crops at the right time and in right quantity for good production and yield. (Olaniyi JO and Ajibola AT, 2008).

Although fertilizers contribute significantly in fulfilling the nutritional requirement of crops, continuous, excessive and deranged use might cause ecological hazards, exhaustion of physio-chemical properties of soil and ultimately lead to poor crop yields. Hence alternate sources of safe fertilizer that enhance crop yields without having adverse effects on soil properties are warranted and to address this, organic farming methods remain the answer. Organic farming maintains the ecological balance and provides stability to the production level without polluting soil, water and air. Organic fertilizers promote better soil structure, supply nutrients, help build up organisms antagonistic to pathogens and are known to improve plant growth (Siddiqui et al., 2004). Moreover, organic fertilizers are known to suppress plant parasitic nematode populations and improve crop tolerance (Siddiqui et al., 2001).

Of the various microorganisms present in the rhizosphere, arbuscular mycorrhizal (AM) fungi are of great value in promoting growth and yield of plants (Siddiqui and Mahmood, 1998). 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. Interaction between AM fungi with a number of plant beneficial bacterial species such as *Azospirillum, Alcaligenes, Arthrobacter*, *Acinetobacter,* etc have been shown to stimulate plant growth in a positive manner. Also, the consequences of co-inoculation of AM fungi and *Azospirillum* in terms of plant growth promotion, root colonization and disease suppression have also been studied.

Hence, recent research studies are being directed towards the synergistic effects of co-inoculation of beneficial microflora. While a plethora of data are available on the studies of co-inoculation of legume crops with bacteria and AM fungi, there are very few reports available on non-leguminous crops. Hence the present study was designed to study the growth and yield response of tomato to co-inoculation of AM fungi with *Azospirillum*.

MATERIALS AND METHODS

The field experiments were conducted during May 2009 to August 2009 at the experimental farm at Annamalai University, Chidambaram.

Preparation of soil and nursery maintenance: The soil used for conducting the pot culture experiment was clay loam in texture with pH 7.3, 195 and 16.5 kg ha^{-1} available N and P_2O_5 respectively**.** Tomato (*Lycopersicon esculantum* Mill) varieties PKM1, 1979 were obtained from Horticulture Farm, Annamalai University and were raised in nursery of one meter square size. 5 kg of farm yard manure was mixed with nursery soil. Nursery bed was supplied with 100 g of 15:15:15 complex fertilizer. Well decomposed farm yard manure, nitrogen through urea, phosphorus through single super phosphate and potassium through muriate of potash as per the recommended doses were used in all the pot culture experiments. The dose of manures and fertilizers per pot were worked out based on the soil weight basis keeping the base of their recommended doses viz., 100:50:35 Kg of N: P: K ha 1 and 20 to FYM ha^{-1} for tomato. Phosphorus application rate however was modified and worked out as per different levels in the pot culture experiment.

Biofertilizer combinations and preparations: The culture *of Glomus fasciculatum* consisting of live AM fungal spores of single species in root tissues of previous host and in soil. The inoculum had an infective propagate of 0.19 x $10^{4} g^{-1}$ and was maintained at 4° C for storage. *Azospirillum vinelandi* and AM fungi *G.fasciculatum* were applied at the rate of 30 g except when modified as per experimental schedule. Fungal culture inoculation was performed by placement method at 25 g of soil inoculum per plant. The tomato seedlings obtained from separate nursery bed were planted at 2 per pot maintaining necessary controls.

Per cent root colonization by AM fungi: Per cent root colonization by AM fungi was determined after staining the roots with tryphan blue as described by Phillips and Hayman (1970) with slight modifications.

Estimation of AM fungal spores in the rhizosphere: The rhizosphere soil was examined for the presence of AM spores by wet sieving and decanting methods described by Gerdemann and Nicolson (1963).

Effect of G. fasciculatum and Azospirillum co-inocuation: A pot culture experiment was set up to study the inoculation effect of *G. fasciculatum* along with *Azospirillum* on growth and yield of tomato and the following treatments were maintained under randomized block design (RBD) with three replicates

T1 - Uninoculated control
T2 - *G. fasciculatum* inoculation only
T3 - *Azospirillum* inoculation only
T4 - *G. fasciculatum* + *Azospirillum* co-inoculation

The biometric observations namely, plant height, plant dry weight, number of fruits per plant, yield per plant, nutrient contents, available soil N and P, percent root colonization and AM fungal spore number per 100 g of soil were determined on 25, 50, 75 and 100 days after transplanting (DAT).

Statistical analysis: The experimental data were statistically analyzed under RBD (Snedecor and Cochran, 1968) using computer aided IRRISTAT package.

RESULTS

Plant growth effects: In both the varieties of tomato, *Azospirillum* and AM co-inoculated plants recorded significantly increased plant height and dry weight than either *Azospirillum* or AM fungi singly inoculated ones. (Table 1)

Table 1. Effects of bio-inoculums on the height and dry weight of tomato crops

S #	Variety	Treatment	Plant height (cm)				Plant dry weight (g)			
			25 DAT	50 DAT	75 DAT	100 DAT	25 DAT	50 DAT	75 DAT	100 DAT
1	1979	Control	12.14	34.85	47.42	57.84	0.85	1.97	2.88	4.03
		+ *Azospirillum*	14.00	31.80	61.89	73.85	1.52	3.42	4.87	6.47
		+ AM fungi	14.94	32.14	62.72	74.25	1.42	3.87	4.93	7.04
		Azospirillum + AM fungi	19.50	37.00	72.12	90.25	2.12	4.01	6.04	8.15
2	PKM 1	Control	12.85	27.24	48.75	60.52	0.85	1.63	3.05	4.12
		+ *Azospirillum*	17.07	34.07	66.15	84.14	1.62	3.52	5.02	6.94
		+ AM fungi	17.14	34.74	67.82	85.63	2.02	3.72	5.55	7.82
		Azospirillum + AM fungi	18.50	36.00	70.00	90.00	2.22	3.85	5.92	8.12
		C.D(p=0.05)	1.32	2.94	4.15	7.60	0.20	0.31	0.35	0.41

DAT – Days after transplanting; C.D – Critical difference; Values – mean of three replications

Table 2. Effects of bio-inoculums on the plant phosphorous content and leaf nitrogen content of tomato crops

S #	Variety	Treatment	Plant phosphorous content (mg plant^{-1})				Leaf nitrogen content (%)			
			25 DAT	50 DAT	75 DAT	100 DAT	25 DAT	50 DAT	75 DAT	100 DAT
1	1979	Control	2.85	6.89	12.02	23.23	2.62	2.75	2.82	2.94
		+ *Azospirillum*	2.92	7.01	12.48	23.86	3.14	3.24	3.82	3.96
		+ AM fungi	4.72	10.88	19.98	42.24	3.42	3.64	4.02	4.15
		Azospirillum + AM fungi	4.92	11.42	20.38	43.24	3.33	3.48	3.94	4.02
2	PKM 1	Control	2.94	6.92	12.09	22.12	2.42	2.65	2.76	2.84
		+ *Azospirillum*	3.02	7.12	12.49	22.62	3.03	3.12	3.74	3.88
		+ AM fungi	4.14	9.22	18.12	35.12	3.34	3.44	3.94	4.02
		Azospirillum + AM fungi	4.87	9.97	19.04	37.19	3.11	3.24	3.78	3.91
		C.D(p=0.05)	0.34	0.71	1.34	2.24	0.25	0.85	1.02	1.98

DAT – Days after transplanting; Values – mean of three replications

Table 3. Effects of bio-inoculums on the total fruit yield (g) in the tomato varieties

S.No.	Tomato variety	Treatments	Total Fruit Yield (g)
1.	1979	Control	984.9
		+ *Azospirillum*	1091.1
		+ AM fungi	1153.8
		Azospirillum + AM fungi	1180.8
2.	PKM 1	Control	900.4
		+ *Azospirillum*	1060.0
		+ AM fungi	1104.3
		Azospirillum + AM fungi	1150.0

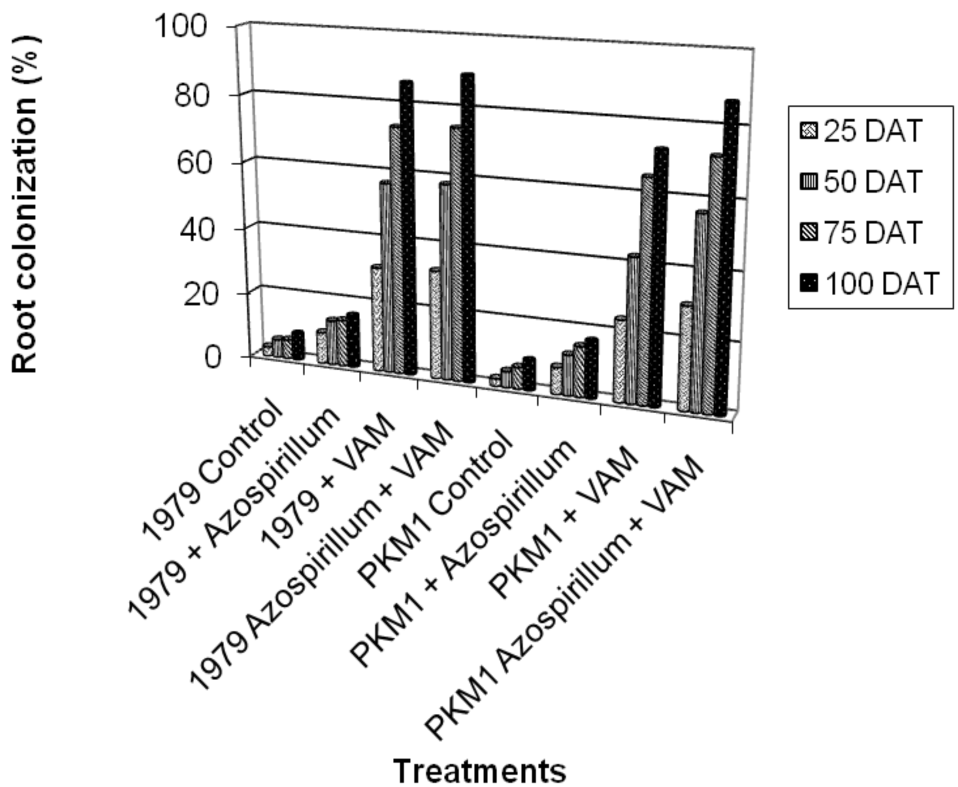

Figure 1. Effect of bio-inoculants on the percent root colonization of tomato varieties

Per cent root colonization: The effect of combination biofertilizers on per cent root colonization in tomato varieties are shown in Figure 1. Compared to the uninoculated plants, the AM fungi inoculated tomato varieties 1979 and PKM1 demonstrated better per cent root colonization. However, maximum root colonization of 89.44 and 86.78 per cent respectively were observed in co-inoculated plant varieties. The per cent root colonization was also observed to increase with the age of plants.

Table 4. Influence of bio-inoculums on the mean acidity, ascorbic acid, lycopene content and T.S.S. of the tomatoes

S#	Tomato variety	Treatments	Mean acidity (mg $100g^{-1}$)	Ascorbic acid (mg 100^{-1})	Lycopene content (mg kg^{-1})	T.S.S. (percent)
1	1979	Control	185	108	58.14	0.23
		+ *Azospirillum*	158	123	63.18	0.26
		+ AM fungi	162	129	68.47	0.29
		Azospirillum + AM fungi	148	140	75.42	0.32
2	PKM 1	Control	190	101	60.00	0.23
		+ *Azospirillum*	160	109	64.62	0.28
		+ AM fungi	165	113	65.04	0.27
		Azospirillum + AM fungi	150	128	72.19	0.33

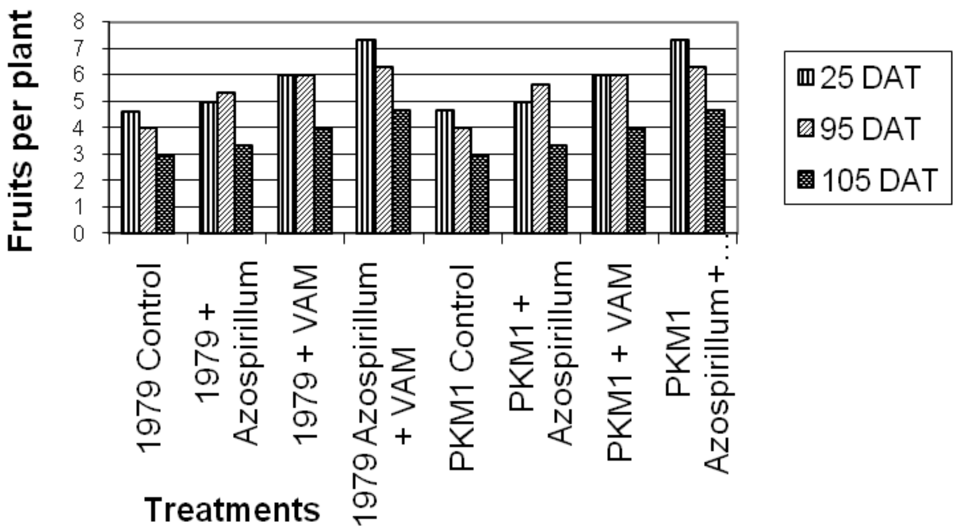

Figure 2. Effect of bio-inoculants on the number of fruits per plant of tomato varieties

Plant phosphorus content and leaf nitrogen content: Significant elevation of plant phosphorus content in both varieties of tomato was observed upon dual inoculation rather than single inoculation (Table 2). The effect of bio-inoculants on plant leaf nitrogen content (percent) of tomato varieties revealed that inoculation of AM or *Azospirillum* singly or in combination increased plant leaf nitrogen content of tomato crop to about 4% over un-inoculated control (2.9 percent). Moreover, when the single inoculums were compared, the AM fungal inoculums brought about substantial increase in nitrogen than *Azospirillum*.

Yield and nutritional content of tomato: Number of fruits per plant significantly increased with single inoculation of either AM fungi or *Azospirillum* than the un-inoculated plants (Figure 2). *However*, the

maximum number of fruits of 4.67 per plant was observed in co-inoculated plants while it was 3.33 fruits for *Azospirillum* and 4 fruits for AM inoculations. The bio-inoculums were also observed to improve the yield of fruits in plants significantly than the un-inoculated plants. (Table 3)
The nutritional content of the tomato fruits such as the ascorbic acid and lycopene content and the acidity of the fruit juices of the varieties of tomato studied were observed to be significantly increased upon co-inoculation with bio fertilizers (Table4). Also, enhanced TSS content of tomato fruits were observed in those plants inoculated with both *Azospirillum* and AM fungi.

DISCUSSION

The extensive research studies on beneficial bacteria and fungi have resulted in the development of a broad range of efficient bio-fertilizers. AM fungi are found in all ecosystems and the plants may be obligatory or facultative dependants on them. AM colonization have been studied to be beneficial to the plants by various mechanisms and among them enhancing the plant- water relations and related physiological characters of the host by improving hydraulic conductivity is paramount (Siddiqui ZA et al., 2007; Mahmood I et al., 2010; Hardie 1985). Many studies have shown that the colonization by mycorrhizal fungi also increases transpiration rate (Bathlenfalvay et al., 1988; Mathur and Vyas 1995). Moreover, AM fungi are also capable of providing substantial protection against nematode diseases (Siddiqui ZA et al., 1999; Waceke *et al.*, 2002; Thygesen *et al.*, 2004).
The AM fungi are the best known for their ability to improve plant growth in low phosphate soils by exploiting large areas of soil and actively transporting the phosphate back to the plants (Siddiqui ZA et al., 2007). Other benefits to the plants supplied by the AM fungi colonization include increased absorption of nitrogen, potassium, magnesium, copper, zinc, boron, sulphur, molybdenum and other elements that are transported back to the plant. Moreover, it helps in retaining moisture around root zone of plants (Siddiqui ZA *et al.*, 2007).
Certain beneficial bacterial flora such as *Azospirillum* are considered a type of biofertilizer that directly help to endow the host plants with nutrients, or indirectly influence positively root growth and morphology, or helps by other beneficial symbiotic relationships (Johansson JF et al.,2004). Among these beneficial activities, facultative root interactions at the rhizospheric level have great implications in ecological and sustainable resource management in agriculture, which include interactions aiding in nutrient exchange, mobilization of exudates/enzymes and modification of root structure (Vessey, 2003, Bohme L and Bohme F, 2006).
Such 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 (Johansson JF et al.,2004), specific bacteria together with AM fungi have been studied to create a more indirect synergism that supports plant growth including nutrient acquisition (Artursson V et al., 2006) inhibition of plant pathogenic fungi, and enhancement of root branching (Gamalero E et al.,, 2004)
The present study revealed that inoculation with AM fungi singly or in combination enhanced plant growth in both the varieties of tomatoes tested. This considerable increase in growth and dry weight could be attributed to the increase in intake of nutrients such as phosphorus, nitrogen, potassium and other micronutrients by the co-inoculated AM fungi and *Azospirillum* and further improved nutrient translocation system. Previous studies in other vegetable crops (Artursson V et al., 2006) have reported significantly increased shoot and root dry weights in when inoculated with AM fungi and grown in nutrient-deficient sandy-loam soils amended with organic matter. Many bacteria have been shown to stimulate plant growth through direct or indirect interactions with plant roots and such flora have been classified as plant growth-promoting rhizobacteria (PGPR). 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

The considerable increase in per cent root colonization in plants co-inoculated with AM fungi and *Azospirillum* observed in the present study could possibly due to the increase intake of nutrients such as phosphorus, nitrogen, potassium and other micronutrients by the synergistic effect of bio-inoculums.

Several studies have unequivocally demonstrated that plants colonized by AM fungi are much more efficient in taking up soil P than non-AM plants (Hudson, 1987).. Results of the present study revealed that inoculation with AM fungi alone brought about substantial increase in phosphorus content and combined inoculation further improved the phosphorus content and yield, thereby establishing the synergistic beneficial activity of two organisms on crop growth.

Previous studies have demonstrated synergistic interactions between phosphate-solubilizing bacteria and AM fungi (Kim KY et al.,, 1998; Artursson V et al., 2006) and have revealed that the bacteria promoted mycorrhizal establishment whereas the mycorrhizal symbiosis increased the size of the phosphate-solubilizing bacterial population. The treatments inoculated with both AM fungi and bacteria significantly increased plant biomass and N and P accumulation in plant tissues, compared with their controls which were not dually inoculated.

The results of the present study also revealed that inoculation with AM fungi or *Azospirillum* singly or in combination increased N content significantly in tomato over un-inoculated controls. While inoculation with AM fungi alone brought about substantial increase in N content, the combined inoculation further improved the growth, phosphorus content and yield. There are also reports of the improvement in nitrogen uptakes caused by AM fungi (Artursson V et al., 2006) and that the increase in nitrogen content is more than that caused by *Azospirillum*. Beneficial effects of *Azospirillum* on plant growth promoting substances besides its main function of N fixation (Artursson V et al., 2006).

Our present study clearly demonstrated that inoculation with AM fungi or *Azospirillum* singly or in combination increased plant growth, and yield significantly in tomato over un-inoculated control. Earlier studies had reported increase in yield parameters of tomato due to inoculation with AM fungi in hydroponics sand culture conditioned (Ojala JC et al., 1980; Mohandas S et al., 1997). In the present study the presence of AM fungi alone brings about substantial increase in growth and yield parameters

Several studies have unequivocally demonstrated that plants colonized by AM fungi are much more efficient in taking up soil P than non-AM fungal plants. Improvement in nutritional status of tomato plants would have resulted in production of higher number of flowers and greater degree of conversion of flowers into fruits. Beneficial effects of AM fungal inoculation in terms of fruit production were more pronounced in combined inoculation. Bagyaraj DJ and Menge JA (1978) observed an increase in rhizosphere population of bacteria and actinomycetes due to dual inoculation of AM fungi and *A.chroococcum*. In the present study, the co-inoculation of AM fungi and *Azospirillum* might have probably acted similarly bringing about an increase growth and yield in tomato plants.

The findings of the present study corroborates with the results of a similar study (Mohandas S et al., 1987), wherein the combined inoculation of AM fungi (*G. fasiculatum*) and *A. vinelandii* brought about an increase in growth and yield in tomato plants.

Similar studies had earlier demonstrated that tomato plants treated with VAM produced significantly higher fruit yield and fruit number compared to the untreated control plants (Utkhede R et al.,, 2006). Mycorrhizal association have been reported to influence the quality of tomato fruit by enhancing ascorbic acid content and reducing acidity. Fruits are often a major sink for phosphorus, and about 65% of phosphorus absorbed by mature plants is transferred to the sink (Chakrabarthy,AK et al., 1990). Accordingly, Mycorrhizal plants could possibly have translocated considerable amounts of mono calcium phosphate to the fruits, which in turn neutralize acidity in fruits. Similar observation of enhanced nutritional status esp. P and N, and improved fruit yield and quality due to AM fungi inoculation was made by Subramanian KS *et al.* (2006).

Thus, the present study consolidates the beneficial effects exerted by the combination of bio-fertilizers on the various factors studied thereby improving the tomato crop production. The synergistic effects of co-inoculation of biofertilizers are found to be superior over single inoculation and hence suggesting a necessity for the balanced bio-fertilizer application in order to derive optimum growth and nutrition of the tomato crops.

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