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Standardization and application of arbuscular mycorrhiza and *Azospirillum* for protray nursery system

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ABSTRACT

Biofertilizers are a sustainable approach to enhance agricultural productivity and environmental health. Arbuscular mycorrhizal fungi (AMF) improve nutrient uptake, particularly phosphorus, and protect plants from pathogens. *Azospirillum* fixes nitrogen, produces hormones and enhances nutrient availability. This study assessed the effect of AMF and *Azospirillum* on the growth, emergence and yield of tomato seedlings in protrays. Results indicate that increasing doses of Arbuscular Mycorrhiza (AM) in cocopeat significantly enhanced shoot and root lengths, germination percentage and seedling vigor, with the highest dose (3.0 kg AM/100 kg cocopeat) showing the best results and maximum root colonization (66.6 %). Similarly, higher doses of *Azospirillum* improved root colonization and seedling growth, with the highest dose achieving the greatest viable count (14.3 × 10 CF Ug $^{-1}$), highest colonization rate (73.3%) and superior growth metrics.

Key words: *Arbuscular mycorrhizal fungi, Azospirillum, Growth and Tomato.*

Biofertilizers are emerging as a valuable alternative to synthetic inputs, offering cost-effective and eco-friendly solutions. They enhance plant nutrition, improve soil quality and boost crop production. Thus reducing reliance on synthetic fertilizers and supporting the agricultural industry's efforts to meet global food demands. By utilizing nutrients naturally present in soil, air and water, biofertilizers complement traditional agrochemicals and contribute to sustainable farming. Arbuscular mycorrhizal fungi (AMF) form symbiotic relationships with about 80 percent of terrestrial plants, enhancing nutrient uptake, particularly phosphorus. They improve plant growth and health by colonizing roots, boosting nutrient acquisition through their hyphae and producing growth-promoting substances. Additionally, AMF protect plants from pathogens and support growth across diverse conditions (Solanki *et al.,* 2021). *Azospirillum* is a key plant growth-promoting bacterium that enhances crop productivity through nitrogen fixation, hormone production and nutrient solubilization. It is widely distributed in various environments and supports agricultural sustainability (Cassan *et al.,* 2020; Pedraza *et al.,* 2020). Protray technology improves seedling production by enhancing germination, root development, uniformity and facilitates easier handling and transportation (Sharmila *et al.,* 2014). Tomato is a major vegetable grown crop worldwide, valued for it's health benefits and plays a significant role in Indian agrarian economy (Jenifer *et al.,* 2022; Chaudhary *et al.,* 2018).

MATERIAL AND METHODS

Glomus mosseae culture was maintained in a polyhouse, using *Pennisetum glaucum* (pearl millet) as the host and vermiculite: perlite: soilrite in the ratio of 3:1:1 by volume + 8% sterilized soil as substrate. The plants were harvested at 75 days after sowing (DAS). Roots were finely chopped along with the substrate, air dried which contained spores and hyphae was used as inoculum. The liquid formulation of *Azospirillum brasilense* was used as inoculum obtained from Agricultural Research Station, Amaravathi, Andhra Pradesh.

Experiment was laid out in a Completely Randomized Design (CRD) comprising of six treatments and each treatment was replicated thrice. Pro trays filled with coco peat were used to test different standardizations of AM (T_1 - Control, T_2 - 1.0 kg AM / 100 kg coco peat, T_3 - 1.5 kg AM /

100 kg coco peat, T4 - 2.0 kg AM / 100 kg coco peat, T5 - 2.5 kg AM / 100 kg coco peat, T6 - 3.0 kg AM / 100 kg coco peat) and *Azospirillum* (T1 - Control, T2 - 50 mL *Azospirillum* / 100 kg coco peat, T3 - 75 mL *Azospirillum* / 100 kg coco peat, T4 - 100 mL *Azospirillum* / 100 kg coco peat, T5 - 125 mL *Azospirillum* / 100 kg coco peat, T6 - 150 mL *Azospirillum* / 100 kg coco peat). Each experiment has six pro trays, with one containing control and the rest inoculated with five different AM and *Azospirillum* treatments. Twenty-one cells in each tray were designated as controls, while eighty-four cells across two trays were used for inoculated treatments. Seeds were sown in planting holes, thinned to one seedling per cell after few days and maintained in a polyhouse with regular watering. The germination percentage was recorded a 15 DAS and seedlings were removed manually from the protray cells and shoot and root length of the seedlings was recorded. The measurements were taken for 15, 30 and 45 days old tomato seedlings for calculation of seedling vigour. Removed seedlings were further used to stain the root segments for determination of AM root colonization and isolation of *Azospirillum.* Coco peat was used for enumeration of microbial population.

Root segments were stained following Philips and Hayman (1970): fixed in FAA solution, cleared with KOH, neutralized with HCL, and stained with 0.05 % Trypan blue in lacto-glycerol for 24 hours, then re-immersed in lacto-glycerol. The percentage of root colonization was determined using the gridline intersect method (Giovannetti and Mosse, 1980), where stained root segments on a gridline glass plate were examined under a stereomicroscope to count the intersections with colonized roots.

$$\text{AM root colonization} = \frac{\text{Total no. of intersections positive for colonization}}{\text{Total no. of intersections between root and gridline}} \times 100$$

Isolation of *Azospirillum* followed the standard procedure by Dobereiner and Day (1976). Fresh root samples were cut into 0.5 cm bits, washed with distilled water, surface sterilized with 0.1 % HgCl, solution for 1 minute and 70 % alcohol 1 minute and then rinsed thoroughly. These sterilized root bits were placed in petri plates with sterilized Nitrogen-free semisolid malate medium, incubated at 30°C for 4-5 days. The presence of subsurface white undulating pellicles indicated *Azospirillum* growth. To determine the *Azospirillum* population, 10 g of coco peat or soil from each sample was mixed with 90 mL of sterile saline water, agitated and serially diluted. A 0.1 mL sample from each dilution (10^{-3}, 10^{-4}, 10^{-5}) was plated on Nitrogen-free malate media and incubated at 28°C ± 2°C for 24-72 hours. The resulting colonies were counted using a digital colony counter and expressed as CFU per gram of soil.

RESULTS AND DISCUSSION

The influence of different doses of Mycorrhiza and *Azospirillum* influence on AM Root colonization, Microbial populaion of *Azospirillum* and seedling growth promotion of tomato in prorays

Effect of AM fungi on seedling growth parameters

The impact of different doses of Arbuscular Mycorrhizal (AM) fungi on tomato seedlings grown in protrays showed significant improvements in shoot length, root length, germination percentage, and seedling vigour. The highest dose of AM in T6 (3.0 kg per 100 kg coco peat) resulted in the greatest shoot length (11.2 cm), root length (7.1 cm), and germination rate (95 %) at 45 days after sowing (DAS). The seedlings in this treatment also exhibited the highest vigour index (1738) at 45 DAS. AM fungi enhance plant growth by extending their hyphae beyond root zones, increasing the surface area for absorption and accessing nutrients in tight soil spaces. AM fungi boost root and shoot length, chlorophyll content, and germination. This symbiotic relationship leads to healthier, more resilient plants and supports sustainable agriculture.

Root colonization

The influence of different doses of Arbuscular Mycorrhizal (AM) fungi on root colonization in tomato seedlings grown in protrays, showed significant results at 45 days after sowing (DAS). The highest root colonization was observed in T6 (3.0 kg AM / 100 kg coco peat) (66.6 %), followed by T5 (2.5 kg AM / 100 kg coco peat) (53.3 %) and T4 (2.0 kg AM / 100 kg coco peat) (44.6 %). The lowest colonization was in the T1 (control) (23.2 %). This trend highlights the positive impact of AM on enhancing root colonization, which is crucial for

Table 1. Influence of different doses of AM on shoot length, root length, germination percentage, seedling vigour and root colonization of tomato seedlings in protrays

Treatments	Shoot length (cm)	Root length (cm)	Germination (%)	Seedling vigour			Root colonization (%)
				15 DAS	30 DAS	45 DAS	
T1	9.8	5.8	70	628	811	1092	23.2
T2	10.1	6	75	691	884	1207	30.3
T3	10.4	6.3	80	758	982	1336	36.4
T4	10.7	6.5	85	823	1068	1462	44.6
T5	10.9	6.8	90	938	1195	1593	53.3
T6	11.2	7.1	95	1198	1311	1738	66.6
SEm±	0.12	0.08	1.34	6.35	8.22	21	0.87
CD(P=0.05)	0.39	0.27	4.23	20	25.9	66.4	2.74
CV (%)	2.06	2.31	2.82	1.31	1.36	2.6	3.56

Treatments
T1 - Control, T2 - 1.0 kg AM / 100 kg coco peat, T3 - 1.5 kg AM / 100 kg coco peat, T4 - 2.0 kg AM / 100 kg coco peat, T5 - 2.5 kg AM / 100 kg coco peat, T6 - 3.0 kg AM / 100 kg coco peat
Note: AM : Arbuscular Mycorrhiza

Table 2. Influence of different doses of *Azospirillum* on shoot length, root length, germination percentage, seedling vigour, total viable count and percentage of root infection of tomato seedlings in protrays

Treatments	Shoot length	Root length	Germination	Seedling vigour			Total viable count of *Azospirillum(× 10-4 CFUg-1 of soil)*	Percentage of root infection by *Azospirillum* (%)
				15	30	45		
	(cm)	(cm)	(%)	DAS	DAS	DAS		
T1	9.7	5.7	69	623	802	1062	3.3	26.6
T2	10.1	6.2	74	687	926	1206	5.6	33.3
T3	10.3	6.4	79	742	978	1319	8.3	40.3
T4	10.5	6.6	84	819	1052	1436	10.6	46.6
T5	11	7	94	1168	1287	1692	14.3	73.3
T6	10.8	6.8	89	907	1158	1566	12.6	66.6
SEm±	0.13	0.11	1.07	17.4	14.5	21.2	0.18	0.74
CD(P=0.05)	0.41	0.36	3.38	54.9	45.8	67	0.59	2.35
CV (%)	2.17	3.1	2.28	3.66	2.43	2.67	3.58	2.71

Treatments
T1- Control, T2 - 50 mL *Azospirillum* / 100 kg coco peat, T2 - 50 mL *Azospirillum* / 100 kg coco peat
T3 - 75 mL *Azospirillum* / 100 kg coco peat , T4 - 100 mL *Azospirillum* / 100 kg coco peat,
T5 - 125 mL *Azospirillum* / 100 kg coco peat, T6 - 150 mL *Azospirillum* / 100 kg coco peat

improving plant growth.

Effect of *Azospirillum* on seedling growth parameters

The application of different doses of *Azospirillum* to tomato seedlings grown in protrays, significantly influenced their growth. At 45 days after sowing, the highest shoot length (11 cm), root length (7 cm), germination rate (94 %) and seedling vigor (1692) were achieved in T5 (125 mL *Azospirillum* / 100 kg coco peat). Lower doses (100 mL and 150 mL *Azospirillum* / 100 kg cocopeat)) also improved these parameters compared to the control, which had the lowest values in all measures. Root exudates, particularly sugars, attract *Azospirillum* through chemotaxis, enhancing root colonization. Inoculation with *Azospirillum* promotes root hair formation, branching, and lateral roots, starting from germination. These bacteria produce growth-promoting hormones and improve nutrient uptake, leading to increased shoot length, root number, root length, leaf area, and plant survival. The effects depend on bacterial age and inoculum level.

Total viable count and percentage of root infection

The study on *Azospirillum* doses in tomato seedlings grown in protrays, revealed significant effects on both total viable count and root infection. At 45 days after sowing, the highest total viable count was observed in T5 (125 mL *Azospirillum* / 100 kg coco peat) (14.3 × 10{ t CFU g{ ¹of soil) in dilution of 10^{-4} then in 10^{-3}, 10^{-5} and the lower counts was observed in lesser doses and in control T1 (3.3 × 10{ t CFU g{ ¹of soil). Similarly, root infection percentage was highest in T5 (125 mL *Azospirillum* / 100 kg coco peat) (73.3 %), showing a marked increase over lower doses and the control T1 (26.6 %). These results highlight the effective colonization and growth- promoting capabilities of *Azospirillum*. *Azospirillum* enhances total viable bacteria by producing growth-promoting substances and attracting rhizosphere bacteria *via* root exudates. *Azospirillum* application significantly improves root colonization and plant growth.

CONCLUSION

Application of both Arbuscular Mycorrhizal (AM) fungi and *Azospirillum* ositively impact tomato seedling growth in a protray nursery. The highest application of AM fungi (3.0 kg per 100 kg cocopeat) and *Azospirillum* (125 mL per 100 kg cocopeat) resulted in significant improvements in shoot and root length, germination percentage, seedling vigor, and overall seedling health. These findings highlight the beneficial role of both treatments in enhancing seedling quality, offering valuable tools for improving plant growth and sustainable nursery management.

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