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Study of diversity of Arbuscular Mycorrhizal Fungi in the rhizosphere of soybean

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The present research examines the diversity and distribution of arbuscular mycorrhizal fungi (AMF) in the rhizosphere soil of soybean across various locations in Satara district, Maharashtra state, India. The findings showed variations in AMF spore populations, root colonization, and the number of AM fungal species among different sampling sites. Sites without irrigation showed higher spore populations and more AM fungal species compared to irrigated sites. A total of 12 AM fungal species from four genera-*Glomus, Acaulospora, Sclerocystis and Entrophospora* were identified, each exhibiting distinct distribution patterns. *Glomus* species were found to be predominant in the soybean rhizosphere soil, followed by *Acaulospora*and*Scutellospora.*

Keywords : *Glomus*, mycorrhiza, rhizosphere, root colonization. spore population

INTRODUCTION

Mycorrhizae represent a mutualistic symbiosis between fungi and the roots of vascular plants. Within this relationship, the fungus inhabits the plant roots, either intracellularly as seen in arbuscular mycorrhizal fungi (AMF) or extracellularly as observed in ectomycorrhizal fungi. These associations are crucial components of soil ecology and influence soil chemistry (B. Sadhana, 2014). Among these associations, arbuscular mycorrhizae formed between plants and Glomeromycota fungi are widely distributed in nature. AM fungi are found in diverse ecosystems such as agricultural lands, forests, grasslands, and various stressed environments, where they colonize the roots of numerous plant taxa including bryophytes, pteridophytes, gymnosperms, and angiosperms.Mycorrhizae are typically classified into ectomycorrhizae, where fungal hyphae do not penetrate individual cells within the roots, and endomycorrhizae, where fungal hyphae penetrate the root cell wall and invaginate the cell membrane. Genera within the AM-forming family include *Acaulospora, Entrophospora, Gigaspora, Glomus, Sclerocystis*, and *Scutellospora*. These fungi enhance plant growth under low fertilityconditions, confer tolerance to certain plant pathogens, improve water balance, contribute to soil structure formation, and aid in plant establishment in new environments (Panwar and Vyas,2002).

Most of the agricultural crops establish mutual beneficial associations with Arbuscular Mycorrhizal fungi, which gives benefit to the host plants directly by enhancing uptake of nutrients that might be physically or chemically inaccessible to root systems, or indirectly by conferring resistance to host plants against biotic and abiotic stresses. (Karima*net al*.2020).

AMF gives fewer benefits of the symbiosis on plant biomass in higher fertility soil (Okiobe *et al*. (2022).However, AMF related benefits extend beyond plant biomass, and include positively affecting plant stability, e.g. improved resistance to stress, and to environmental quality, e.g. by reducing leaching and promoting soil aggregation.

This study aimed to investigate the natural diversity of AM fungal spores in soybean

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rhizosphere soil and their colonization patterns in relation to local variations across different sites in Satara district, Maharashtra state, India.

MATERIALS AND METHODS

Location of sampling sites

The study was conducted in Satara district, Maharashtra state, India, encompassing both irrigated sites such as Karad and Patan, and non-irrigated sites including Man and Khatav. Satara district is situated between 17°05' to 18°01' North latitude and 73°03' to 74°05' East longitude, covering a geographical area of approximately 10,480 sq.km. The region experiences an annual rainfall of 1042 mm / (41 inches) and average temperature ranging from 23°C to 37°C (74°F). The average humidity ranges from 21% to 99%.

Collection of root and soil samples

Soil samples and fine roots were collected in polythene bags from various locations. Approximately 500 to 1000 g of soil from the rhizosphere of each plant at a depth of 15-30 cm were collected and combined to create composite samples. These composite samples were transported to the laboratory for the isolation of AM fungal spores, quantification of mycorrhizae, and assessment of root colonization, and then stored at 5-10°C. Fine roots were separated from the samples, washed with tap water, and preserved in formalin for root colonization analysis. The soil samples were air dried in the shade at the laboratory for further analysis.

Isolation of AM fungi from soil

Wet sieving and Decanting method were used for isolation of AM fungi spores(Dwivedi, 2008). For this sieve of different sizes i.e. 300,150, 75 and 30 µm were used. 50 gm of soil were dissolved in 500 ml of water in a beaker using stirrer and allowed to settle. The sieves were placed in the following order 300,150, 75 and 30 µmfrom top to bottom. The water of the beaker was decanted through the series of the sieves, on which spores were trapped and then were washed with running tap water. The trapped spores were transferred to Whatman No. 1 filter paper by repeated washing with water. Spores were picked using a needle under stereo binocular microscope. The spores were mounted on glycerol for further observation.

Mycorrhizal quantification

Quantitative estimation of AM fungi spores was done by modified Grid line intersect method. (Chatterjee*et al*. 2010). In this method, the filter paper was divided into small compartments by a ball point pen and each compartment was numbered. The total numbers of spores were counted under stereo binocular microscope.

Determination of root colonization

Freshly collected roots were washed gently to free from soil particles. Roots were treated with 10% KOH solution for 30 mins on a hot plate. The treated roots were washed with water and treated with 2% HOCL solution. Acidified roots samples were stained with 0.05% trypan blue in lactic acid for overnight. These roots were observed under compound microscope. Fungal structure is stained and can be easily observed. (Okiobe*et al*. 2022). The mycorrhizal colonization was determined by using the following formula.

$$\text{Root colonization (\%)} = \frac{\text{Number of AM positive segments}}{\text{Total number of segments observed}} \times 100$$

Identification of AM fungi

The main structure of AM fungi, the spores were used for identification. Following morphological characteristics viz; colour, size, shape, wall structure, bulbous suspensor, the number and arrangement of spores in the sporocarp were used for AM fungi identification. These AM fungal spore were identified with the help of Schenck, Walker, Perez and Mukerji (Kryukov *et al*. 2020).

Statistical analysis

Ecological measures of diversity used to describe the structure of AMF communities included spore density, species richness, relative abundance, isolation frequency, Spore density reflects the biomass of AMF species, which indicated the sporulation ability of different species

of AMF. Isolation frequency was defined as the percentage of soil samples in which a species occurred, which revealed extent of distribution of given AMF species in ecosystem. spore density and Species Richness, Relative abundance was reflected by distribution of VAM species.

RESULTS AND DISCUSSION

Diversity measures of AM fungi are provided in Table 1. The rate of AM fungal colonization and VAM spore diversity in rhizosphere of soybean showed variation in different sites of selected localities. Maximum abundance of AM spore was recorded in non – irrigated sites than irrigated sites. A total of 12 localities,730 spores of AMF belonging to 12 AM fungal species were isolated by wet sieving and decanting method from collected soil samples of different sites. Of the isolated AM spores *Glomus* was represented the dominant genus followed by *Acaulospora, Sclerocystis* and *Entrophospora*. The result of present study supports the findings given by (Sharma*et al*. 2008). who reported the dominance of the *Glomus*. They described that the wider adaptation of this taxon in varied soil conditions may be attributed to the sporulation pattern of *Glomus*. *Glomus* species are considered as cosmopolitan fungi in many ecosystems. (Skykorova*et al*.2007). They usually occur in neutral and slightly alkaline soil. (Mukerji *et al*.2013) Other genera like *Scutellospora* and *Gigaspora* are less common in present study. There were only small number of Species present in Gigasporaceae.

Relative abundance of isolated AM are given in Table 2 while Table 3 reveals spore density. It was observed that *Glomushalon* (9.04 % of RA) was dominant followed by *Glomus deserticola*(8.49 %of RA) and *Glomus microcarpum* (7.80 % of RA). Table4 represents that percentage of AMF root colonization of soybean are greatly varied at different sites. Highest infection 90% was observed in F1 of non- irrigated site than 30% found in F12 from irrigated site. More AMF root colonization percentage found in non-irrigated site of soybean than irrigated site. In this investigation

Table 2: Relative abundance of rhizosphere soil of soybean

Name of the AMF spore	Relative Abundance (RA%)
Acaulospora undulata	1.09
Acaulospora laculosa	1.50
Entrophospora sp.	0.82
Glomus etunicatum	6.57
Glomus radiatum	4.65
Glomus microcarpum	7.80
Glomus austral	3.42
Glomus deserticola	8.49
Glomus claoides	7.94
Glomus halon	9.04
Glomus fasciculatum	5.06
Sclerocystis sp.	1.23

Table 3 : The AMF spore density of different sites

Locality	Study site	Spore count
Non – Irrigated site (NIR)	F1	56
1.Man	F2	48
	F3	67
2.Khatav	F4	78
	F5	72
	F6	86
Irrigated site (IR)	F7	46
1.Karad	F8	61
	F9	34
2.Patan	F10	54
	F11	70
	F12	58
Total spores	730	

Table 1: Diversity measures

Spore density (SD)	The number of spores in 100 gm of soil
Species richness (SR)	Number of identified AMF species per soil sample
Relative abundance (RA)	$\frac{\text{Spore number of species}}{\text{Total number of identified spore samples}} \times 100$

Table 4 : Percentage of root colonization of AMF in roots of Soybean

Locality	Study site	% of AMF root colonization	Number of vesicles per root	Number of arbuscules per root
Non- irrigated site	F1	90	6	4
1.Man				
	F2	70	3	3
	F3	60	4	1
2.Khatav	F4	80	5	2
	F5	60	4	1
	F6	70	3	2
Irrigated sites	F7	40	2	4
3.Karad	F8	50	4	1
	F9	80	3	3
4.Patan	F10	70	1	1
	F11	60	4	1
	F12	30	2	3

SR was maximum at F6 9% (SD 86/100g soil) followed by F4 9% (SD 78/100g soil). Spore density and species richness is found minimum in F9 (SD 34/100g soil) and F7 (SD 46/100g soil). Photographs of isolated AM fungal spores and root colonization are provided in Fig. 1 & 2.

CONCLUSION

The present investigation shows good association of AM fungi in soybean. It also shows the fact, that this symbiosis controlled various edaphic factors. Highest infection of AMF root colonization indicates better fungal root contact which is pre requisite for increased benefits of AMF symbiosis and better adaptation to present soil. The present study shows increasing AMF spore density in non-irrigated sites than irrigated sites. *Glomus* sp. was dominant in all sites. The soybean plant offers the possibility if using AM fungi as a Potential bio-fertilizer for enhancement of crop growth and productivity. Mycorrhizal inoculants can be used to boost the overall plant growth and is a great tool for progressive farmers who are focusing on sustainability of agriculture and are keen in making improvements in their farming practices.

DECLARATION

Conflict of interest: Authors declare no conflict of interest.

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Table 5: Diversity measurement of AMF Population in present study

Locality	Spore Density/100g of soil (SD)	Species Richness (SR)
Non – irrigated sites		
1.Man		
F1	56	8
F2	48	7
F3	67	5
2. Khatav		
F4	78	9
F5	72	6
F6	86	10
Irrigated sites		
1.Karad		
F7	46	4
F8	61	7
F9	34	3
2.Patan		
F10	54	5
F11	70	8
F12	58	6

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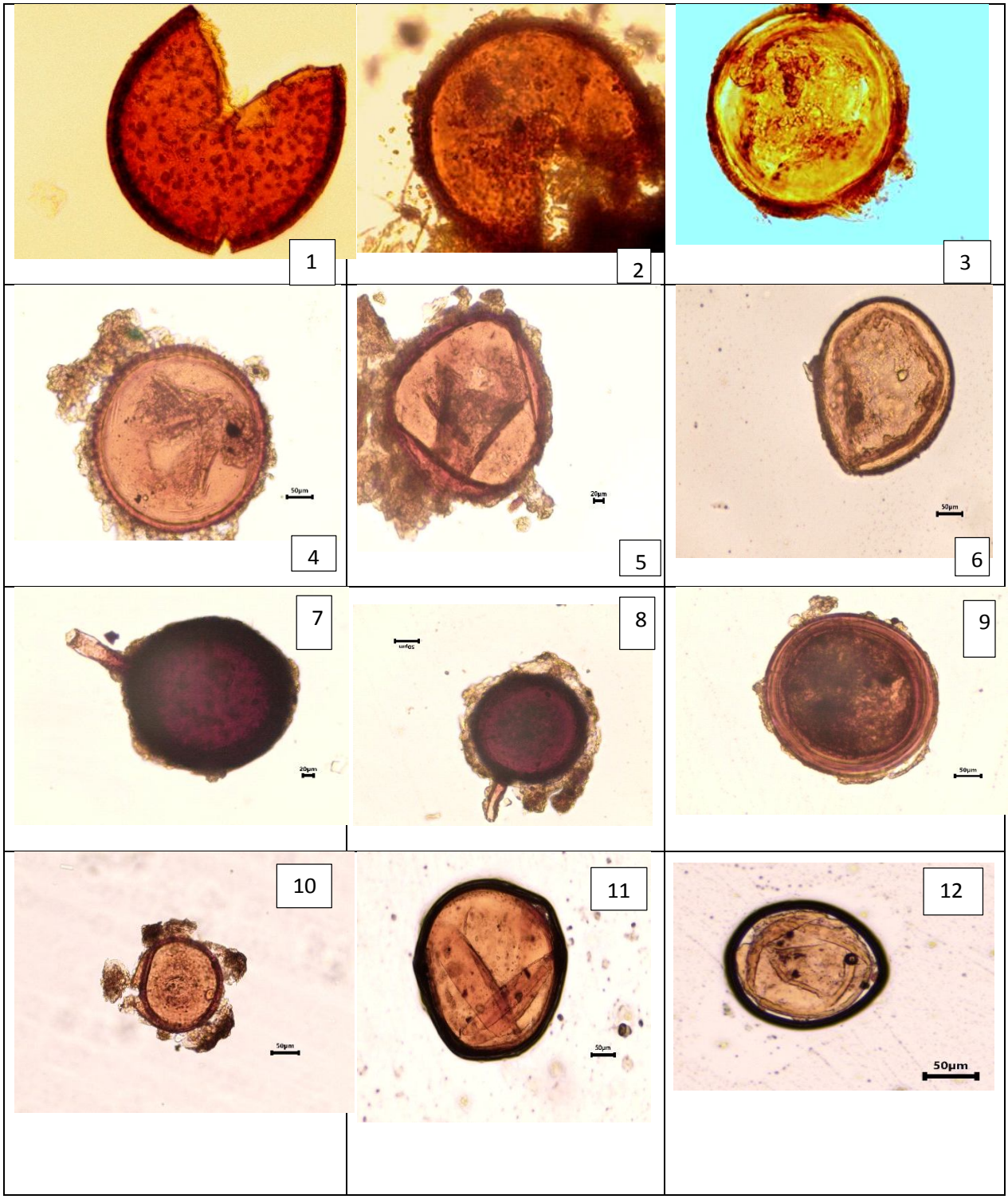

Fig. 1: AMF spores. 1.Acaulospora undulata 2. Glomus etunicatum 3. G. radiatum 4. G. microcarpum 5. G. australe 6. Scleosyctis sp. -7 Entrophospora sp. 8. G. deserticola 9.G. claoides 10. A. laculosa 11. G. halon 12. G. fasciculatum

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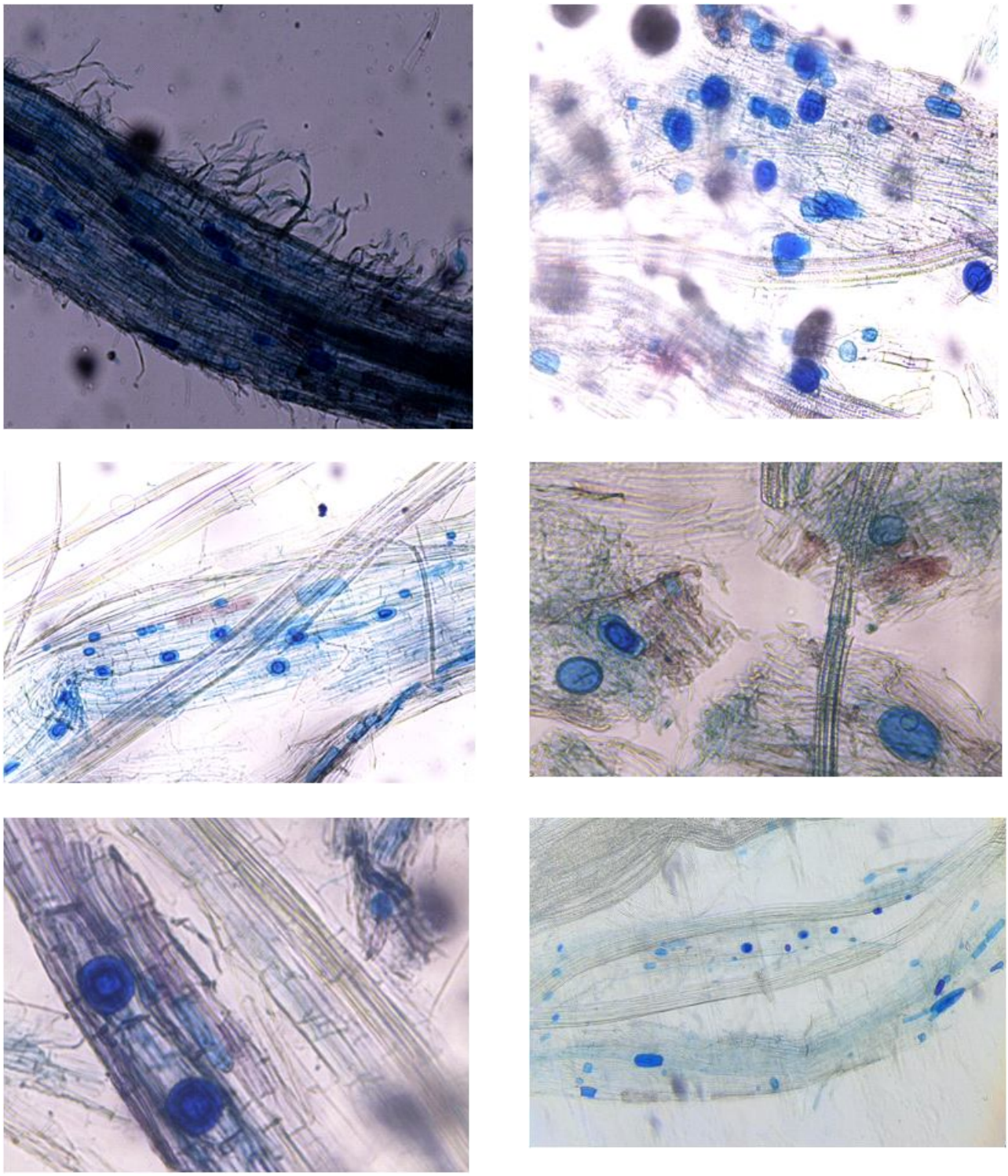

Fig. 2 : Root Colonization by AM fungi

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