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Role of Arbuscular Mycorrhizal Fungi in plant health and Seasonal comparative analysis of its occurrence on some selected plant species

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Abstract
The present paper deals with the beneficial role of arbuscular mycorrhizal fungi in plant health and study of seasonal analysis of occurrence of arbuscular mycorrhizal fungi in the root and rhizospheric soil of some selected plants. The plants were collected on seasonal basis and all collected plant species showed mycorhizal infection. Maximum root colonization was recorded in summer season and minimum in rainy season. The rhizospheric soils of the collected plant species were with AM fungal spores found highest in winter season while lowest in summer season. The percentage of colonization was varied with plant species. The AMF genus from the site noticed was genus *Glomus, Acaulospora, Enterospora, Gigaspora* etc with maximum number of species. Glomeromycota was the fungi found to be dominating in present work.

Keywords: Arbuscular, colonization, Glomeromycota

1. INTRODUCTION:

Mycorrhizae are a lead soil microbial component that plays a vital role in recovering deteriorated ecosystems [1] Nearly 80% of terrestrial plants develops useful plant growth responses as a result of its symbiotic association with mycorrhizal (AMF) Arbuscular mycorrhizal fungi [2]. Arbuscular mycorrhizal fungi are the most common form of symbiosis. The arbuscular mycorrhizal fungi (AMF) are observed to be present all over the world particularly in the soil. The arbuscles are considered the major site of nutrient transfer to the plant [3,4]. AMF facilitates host plants to grow vigorously under stressful conditions leading to enhanced photosynthetic rate, increased water uptake, and gas exchange-related traits [5] AMF forms vesicles, arbuscles, hyphae in roots, and spores and hyphae in the rhizosphere. Different kinds of spores, like chlamydospores, zygospores, etc. These spores were observed to remain in contact with a large number of plant communities like Bryophytes, Pteridophytes, Gymnosperms, Angiosperm's from all over the geographical areas [6] Plant growth is upgraded due to the formation of a hyphal network by AMF with plant roots, as this notably increases the access of roots to a large soil surface area [7]. The plant health is improved

by AMF as it refines the quality of soil structure and texture [8,9]. The plants with Vesicular Arbuscular mycorrhiza (VAM) can more efficiently absorb Potassium, Iron, Copper, and Calcium, they accelerate water uptake. They can grow in less irrigated lands and with less supply of chemical fertilizers, thus the association of VAM with crop plants helps in the conversion of less-productive fields into more productive fields. In this mycorrhizal association, both arbuscular and vesicles formed together which is the most common type of mycorrhizal association found in more than 90% of plants. When the mycelium of mycorrhiza lives in between and within the cortical cells of the roots, then they are termed endo-mycorrhiza. Mostly the endomycorrhiza is found in the herbaceous species and some woody plants. It is noticeable that the mycorrhizal fungi have many important functions in ecosystems [10] Therefore the aim of the present study is to list arbuscular mycorrhizal fungal species in the rhizosphere soil samples of the selected plant species in, Maharashtra, and seasonal comparative analysis of their occurrence.

2. ROLE OF ARBUSCULAR MYCORRHIZAL FUNGI

The arbuscular mycorrhizal fungi symbiosis with plants had been reported 400 million years ago [11] this association is a classical example of a mutualistic relationship that controls the growth and development of plants. They are considered as natural growth regulators of the majority of terrestrial flora and used as well known bio-fertilizers in sustainable agriculture [12] they are soil-borne fungi and greatly improve water and mineral nutrient uptake [13] they also safeguard the plants from fungal pathogen [14] they play a lead role in sustainable crop improvement [15]

Role as a Bio-fertilizer

Naturally occuring substances used to improve soil fertility are termed biofertilizers. The use of inorganic fertilizers causes various problems to the soil, plants, and human health, impacting the quality of food products.It is widely accepted by different research studies that AMF could be considered as a substitute of inorganic fertilizers in near future and possibly lower down the use of chemical fertilizers upto 50% for best agricultural production.

Role in Mineral nutrition

AMF can boost the uptake of inorganic nutrients in almost all plants, specifically phosphate [16]. Their association has been reported to increase the phyto-availability of micronutrients like zinc and copper.Fungal structures like arbuscles are produced by AMF which facilitate the exchange of inorganic minerals and compounds of carbon and phosphorus,eventually imparting vigor to host plants[17]

Role in Plant Yield

The quality of crops is enhanced by microorganisms inhabiting the rhizosphere. The dietary quality of crops is intensified by AMF. The sugar content, vitamin C, flavonoids, and mineral content was increased in citrus fruits due to *Glomus versiforme* **[**18**]** AMF also adds value to phytochemicals in edible plants making them good enough for a healthy food production chain

Role in Abiotic stress

Plant life is impacted in many ways due to drought stress, it imparts damaging effects on the growth of plants by affecting enzyme activity, uptake of ions, and nutrients [19] The symbiotic association of various plants with AMF improved the root size, leaf area index and biomass under immediate conditions of drought [20] its symbiosis results in increased gas exchange, transpiration rate and stomatal conductance [21]. Studies reported the effectiveness of AMF contribution to growth and yield in plants under salinity stress [22].

Heavy metals- AMF extensively supports plant formation in soils contaminated with heavy metals. There are many reports on detecting the AMF-induced effects on the build-up of metals in plants [23] Heavy metals may accumulate in food crops, fruits, vegetables, and soils causing various health hazards [24] The fungal hyphae originate in soil internally and externally can fix heavy metals by immobilizing them [25].They fix them in the cell wall or store them in the vacuole [26] Thus AMF are critical for improving plant growth and yield under stress.

3. MATERIAL AND METHODS

Sample collection

Totally 11 plant species belonging to 9 families were collected from the Maharashtra region The roots of the plants were collected along with the rhizospheric soil sample of widely growing plants, without any harm to the root system. The collected samples were transported to the laboratory immediately after collection.

Root samples

Root samples were collected from the plant species they were washed thoroughly with tap water until the removal of adhering soil. The washed roots were cut into 1cm length each. For identification and nomenclature of the plant species, the following manual was used [27, 28]

Soil samples

The rhizosphere soils were collected from each plant species by drilling up to 10 cm depth. These samples were kept in sterilized bags and were transferred to the laboratory immediately for the arbuscular mycorrhizal fungal spore isolation.

Soil pH

pH meter was used to determine the pH of soil samples (soil-water suspensions (1:5)

Assessment of arbuscular mycorrhizal colonization in roots

The arbuscular mycorrhizal root colonization was studied by using the method suggested by [29]. The root segments were kept in 10% KOH and autoclaved for 15 minutes at the proper pressure. After that KOH was removed and dipped these root segments into 1N HCL for 5 minutes. After that root segments were stained in cotton blue with lactophenol overnight. In next day such stained root segments were screened under a microscope, for their arbuscular mycorrhizal root colonization. The whole mount of root segments showed the presence of arbuscles, vesicles, and mycelium as the roots were positively mycorrhizal with colonization. The percentage of root colonization was calculated by using the following formula [30].

$$\text{Percentage of root colonization} = \frac{\text{Numberofrootsegmentscolonized}}{\text{Totalnumberofrootsegmentsscreened}} \text{ X100}$$

Along with the calculation of root colonization, the air-dried rhizospheric soil sample was preserved for the study of the spore population. Isolation of spore was done by using the method of [31] 100 gm of air-dried rhizospheric soil dissolved in water with a few drops of tween-20. The above solution was continually stirred with a glass rod and finally allowed to settle down for about 20 to 25 minutes. Such settled solution then sieved through different sized sieves as 710mμ, 210mμ, 150mμ, 75mμ, 45mμ, 25mμ respectively. The content of each sieve is now poured into separate Petri dishes for examination of spores. Total spore count was calculated by counting the spores. Then spores were separated using a glass pipette. The spores were mounted on glass slides using lactophenol,covered with coverslips and sealed with DPX medium.

Identification of AM fungi

AM fungal spores were identified based upon microscopic characters. For identification and nomenclature, keys were used by [32, 33, 34] they were classified based on color, size, surface, structure, nature of spore contents hyphal attachment.

4. RESULTS

AM fungal infection and spore population of 11 plant species of 9 families are present in Table 1, it also presents the pH of the rhizospheric soil samples that was ranging between 5.8 to 6.9. In the present study, total 17 AM fungal species belonging to 7 genre were identified. Seasonal comparision of there hyphal colonization, arbuscular, vesicular root colonization, distribution of spore population and physico-chemical properties were studied.

Table 1. Plants with arbuscular mycorrhizal fungal spore population and root colonization

Sr no.	Plant name	Family	Habits	Soil pH	Type of colonization	Root colonization in %	Spore population /100gm soil
1	*Biophytim sensitivum L.*	Oxalidaceae	Herb	6.9	HVA	29.1	800
2	*Lophopogon tridentatus*	Poaceae	Herb	6.8	HA	37.23	1040
3	*Desmodiumdic hotomum Wild*	Fabaceae	Herb	6.7	HV	27.7	760
4	*Indigofera linifolia L.*	Fabaceae	Herb	5.8	HVA	38.1	600
5	*Crotalaria hebecarpa Dc.*	Fabaceae	Herb	6.2	--	0	720
6	*Tricodesma indicum*	Boraginaceae	Herb	6.7	HV	26.8	560
7	*Polycarpeacorymbosa L.*	Caryophyllaceae	Herb	5.9	HVA	36.6	840
8	*Stylosanthes fruticosa*	Fabaceae	Shrub	6.4	HVA	35.2	960
9	*Commelina banghalensis L.*	Commelinaceae	Herb	6.5	HVA	28.33	760
10	*Euphorbia heterophylla L.*	Euphorbiaceae	Herb	6.7	HA	18.3	960
11	*Lantana camara L.*	Verbenaceae	shrub	6.9	HVA	31.43	--

H- Hyphae, A- Arbuscules, V- Vesicle, --Absent

Table 2. Distribution of AM fungal spores of different plant species

Sr. no.	Plant name	Family	Am fungal species
1	*Biophytimsensitivum L.*	Oxalidaceae	*Acaulospora thomii, Acaulospora foveata, Gigaspora decipioens, Glomus microcarpum*
2	*Lophopogontridentatus*	Poaceae	*Acaulospora gadanskensis, Acaulospora delicate, Entrophopora infrequens, Glomus lacteum, Glomus maculosum*
3	*Desmodiumdichotomum Wild*	Fabaceae	*Acaulospora thomii, Acaulospora delicate, Gigaspora decipioens, Glomus aggregatum*
4	*Indigoferalinifolia L.*	Fabaceae	*Acaulospora laevis, Entrophopora infrequent, Glomus glomerulatum, Glomus fragilistratum,*
5	*Crotalaria hebecarpa Dc.*	Fabaceae	*Acaulospora spinose, Acaulospora foveata, Gigaspora rosea*

6	*Tricodesmaindicum*	Boraginaceae	*Entrophopora infrequens. Gigaspora rosea, Glomus glomerulatum, Glomus fragilistratum,*
7	*Polycarpeacorymbosa L.*	Caryophyllaceae	*Acaulospora foveata, Acaulospora gadanskensis, Gigaspora rosea*
8	Stylosanthesfruticosa	Fabaceae	*Acaulospora spinose, Acaulosporal aevis, Glomus maculosum, Glomus aggregatum*
9	*Commelinabanghalensis L.*	Commelinaceae	*Acaulospora foveata, Acaulospora thomii,Entrophopora infrequens, Glomus dimorphicum*
10	*Euphorbia heterophylla L.*	Euphorbiaceae	*Acaulospora gadanskensis, Gigaspora decipioens, Glomus lacteum, Glomus dimorphicum*
11	*Lantana camara L.*	Verbenaceae	*Entrophoporainfrequens, Acaulosporalaevis, Glomus aggregatum, Glomus flavisporum,*

Table 3. Seasonal distribution of of mycorrhizal fungal spores

Season	**Spores**					
	Acaulospora	Archaeospora	Entrophospora	Gigaspora	Glomus	Scutellispora
Rainy	**ATHM,AGDN**	-	-	**GDCP**	**LAGR,LMRC, LFGS**	-
Winter	**AFVT, ADLC, ASPN**	-	**EIFQ**	**GDCP, GRSA**	**LGML,LMCL,LFLS, LLCT,LDMR**	-
Summer	**ALVS**	-	-	-	**LAGR,LMRC**	-

ATHM- *Acaulospora thomii*, **AGDN**- *Acaulospora gadanskensis*, **AFVT**- *Acaulospora foveata*, **ADLC**- *Acaulospora delicate*, **ASPN**- *Acaulospora spinosa*, **ALVS**- *Acaulospora laevis* **EIFQ**- *Entrophoporain frequens*, **GDCP**- *Gigaspora decipioens*, **GRSA**- *Gigaspora rosea*, **LAGR**- *Glomus aggregatum*, **LMRC**- *Glomus microcarpum*, **LFGS**- *Glomus fragilistratum*, **LGML**- *Glomus glomerulatum*, **LMCL**- *Glomus maculosum* , **LFLS**- *Glomus flavisporum*, **LLCT**- *Glomus lacteum*, **LDMR**- *Glomus dimorphicum*, - ***Absent.***

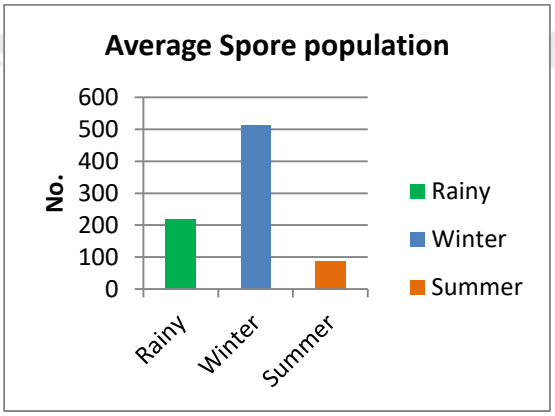
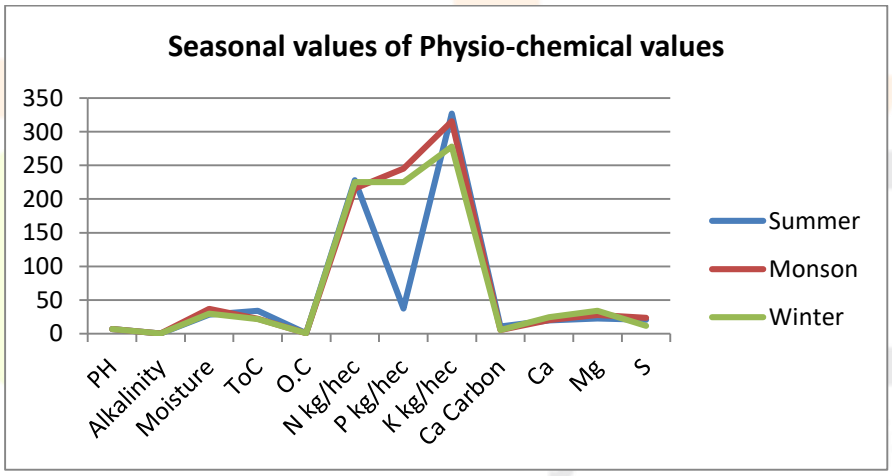


Table 4.Seasonal Comparison of Physico-chemical properties

Physio-chemical property		Summer	Monsoon	Winter	P value
PH	Mean	6.94	6.68	6.80	P<0.01
	SD	0.17	0.16	0.10	
Alkalinity	Mean	0.14	0.18	0.12	P<0.01
	SD	0.01	0.02	0.01	
Moisture	Mean	28.26	36.56	29.50	P<0.01
	SD	0.21	0.05	0.12	
Temperature	Mean	33.96	21.94	21.58	P<0.01
	SD	0.13	0.55	0.40	
Organic carbon	Mean	0.46	0.47	0.66	P<0.01
	SD	0.03	0.02	0.01	
Nitrogen Kg/hec	Mean	228.00	215.60	225.20	P<0.01
	SD	1.22	5.18	0.45	
Phosphorus Kg/hec	Mean	37.16	245.00	225.20	P<0.01
	SD	5.78	1.22	0.84	
Potassium Kg/hec	Mean	327.20	315.60	278.20	P<0.01
	SD	3.03	1.34	0.45	
Calcium Carbon	Mean	10.84	5.24	5.44	P<0.01
	SD	0.68	0.21	0.15	
Calcium	Mean	19.70	19.98	24.40	P<0.01
	SD	0.42	0.61	0.10	
Magnesium	Mean	22.72	27.82	34.14	P<0.01
	SD	0.38	0.48	0.49	
Sulphur	Mean	20.40	23.40	11.60	P<0.01
	SD	1.34	1.67	0.55	



The variations in physico-chemical properties were studied during summer, Monsoon and winter season. Statistically highly significant difference observed in each physico-chemical property (P<0.01)

5. DISCUSSION

This paper focuses mainly on the root infection with hyphae, arbuscules, and vesicles along with the number of AM fungal spores and the study of rhizospheric soils of collected plant samples. Mycelial, arbuscules, and vesicular root colonization were differed not only seasonally but also from plant species to species. AMF root infection was found highest during the summer and winter

season followed by the rainy season. All studied plants were found to be mycorrhizal with hyphal root colonization. In most of the selected plant species, mycelia were present along with vesicles, arbuscules but it seems to be varied with seasons. Hyphal root colonization was found maximum during the winter and summer season while minimum during the rainy season. The hyphal colonization ranged from almost 20% to 68%. In the rainy season, hyphal colonization was very rare recorded from collected plant species, of this *Lantana camera* L. (Verbenaceae) was with the highest hyphal colonization 32.3%. In winter maximum hyphal colonization was in *Stylosanthes fruticose* (Fabaceae) with 55.7% and it was maximum during the dry season in *Indigofera linifolia* L. (Fabaceae) with 67.75%. The presence of arbuscules was not found to be equal in all collected plant species and also in some plants arbuscules were found to be completely absent. AMF colonization was found lowest in the rainy season while highest during the winter and summer seasons. In the rainy season, maximum arbuscular colonization was in *Lophopogon tridentatus* (Poaceae) with 42.35%, while *Indigofera linifolia* L. (Fabaceae) was with 35.35% arbuscular colonization during the winter season. *Lophopogon tridentatus* was with 37.75% arbuscular root colonization during the summer season. Vesicular root colonization was found to be completely absent during the rainy season as compared to the winter and summer seasons. The presence of maximum and minimum vesicular colonization in different plant species in the different seasons was depicted in Table 1. In the winter season, maximum vesicular colonization was in *Commelina banghalensis* L. (Commelinaceae) while during the summer season it was in *Indigofera linifolia* L. (Fabaceae). Vesicular colonization was maximum during the summer and winter seasons which might be due to the favorable condition for plant respiration and oxygen diffusion rate in the soil zone.

From the same site, the AMF spore population was compared in all three seasons,
Lophopogon tridentatus of Poaceae (440spores/100gm soil) in summer season shows maximum spore population while *Euphorbia heterophylla*L. Euphorbiaceae shows minimum spore density i.e. (180 spore/ 100gm soil) during summer. *Polycarpea corymbosa* L. Caryophyllaceae shows maximum spore population (1,040 spore/100gm of soil) while *Indigofera linifolia* L. Fabaceae shows minimum sporulation i.e. (560/100gm soil) during the winter season. It means that there is no such correlation between the percentage of root colonization and spore population. From the site of the collected plant species, the genus Glomus was found dominant with eight species followed by Acaulospora, Gigaspora, and Entrospora. Scutellospora were found absent.
Glomeromycota was the fungi found to be dominating in the present work.

CONCLUSION AND FUTURE RESEARCH

In this article existing information on the beneficial role of AMF combined with experimental research on some AMF symbiotic association with a variety of plants in different seasons is presented. The use of AMF is to be uplifted due to its huge significance in agricultural improvement. AMF reduces the usage of synthetic fertilizers and other chemicals promoting biologically healthy agriculture.
Genes and gene products controlling the growth and development of plants under stressful conditions mediated by AMF need to be linked. The protein factors controlling symbiotic association of both hosts and AMF to be recognized. Identification of the major cellular and metabolic pathways under different environmental stresses are areas for future research in this field.

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