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Seasonal Diversity of Arbuscular Mycorrhizal Fungi (amf) in Marsilea Quadrifolia Pteridophyte from Naldurg Region of Maharashtra

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ABSTRACT

Mycorrhizal colonization of *Marsilea quadrifolia* L. plant was investigated for three seasons. Sixteen soil parameters were analyzed & results revealed that alkaline with pH of 7.71. Electrical Conductivity (EC), Phosphorus (P) Boron (B) and Sodium (Na) were very poor indicating that there are salinity and plant growth problems. The results revealed that the rejects were more in Organic Carbon (OC), Nitrogen (N), Potassium (K), Calcium (Ca), Magnesium (Mn), and sulphur (S). Zinc (Zn), Ferrous (Fe) and Molybdenum (Mo) is favorable for the plant growth. Percentage of Arbuscular mycorrhizal (AM) infection, number of resting spores and AM fungi species varied in all three seasons. Among all three seasons spore density and percentage of root colonization was found more in the winter season. Spore density was found more in winter season (425±12.33/100 g soil) and less than monsoon season (03±1.11/100g soil) followed by summer. Among AMF spore viz. *Sclerocystis, Glomus,* and *Gigaspora* genus was found but *Glomus* found dominant.AMF percentage of root colonization was increased in winter season (35.41±5.11%) while less in monsoon (16.66±2.11%). AMF root colonization types are arbuscular (A), vesicular (V) hyphal (H) and DSE were observed in all season but vesicular and arbuscular types are absent.

1. Introduction

Marsilea quadrifolia L. (water clover, four leaf clover, water shamrock), with its unusual and remarkable leaves for their simplicity is an aquatic and amphibious fern that belongs to Marsileaceae family. *Marsilea quadrifolia* is considered the lectotype of the generic name (1). It is the unique representative of *Marsilea* species are easily recognized by their clover like leaves, which are composed of four leaflets on a long petiole. *Marsilea* is found primarily in seasonally wet habitats, where it grows in shallow water and at the edges of ponds, lakes, or rivers (2).*Marsilea quadrifolia* is spread in Central and Southern Europe, the Caucasus, Central Asia as well as Japan,Siberia, India. In North America it has been established in the northeastern United States for over 100 years (3). It is invasive in natural and disturbed wetland habitats from New England to Florida (4). Campaigns against the spread of this species in natural aquatic habitats are taken in the U.S.A because in New World, *Marsilea quadrifolia* is evaluated as an aggressive and invasive non-indigenous species (5, 6, 7).The AM Fungi is ubiquitous group of fungi (8). AM Fungi belongs to phylum Glomeromycota (9). AMF have been shown to differentially colonize plant roots, causing a variety of effects on plant growth, biomass allocation and photosynthesis (10). *Marsilea quadrifolia* L. out of 14 species of reported from India (11,12), VAM association has been recorded on *M. quadrifoliata* L. (13). The present report is on the association of AMF fungi with the roots and rhizomes of *M. quadrifoliata*. The studies on VAM have been reported in aquatic pteridophytes (14). The endomycorrhizal association is of general, but sporadic and variable, occurrence in all families except the aquatic Marsileaceae, Salviniaceae and Isoetaceae (15). Therefore the present investigation was made to evaluate the AMF status of *Marsilea quadrifolia* in different seasons from Naldurg region of Maharashtra.

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2. Materials and Methods

Soil Analysis: For soil analysis, rhizosphere soils of *Marsilea quadrifolia* plant was collected from 3 different seasons of same locality (Naldurg Region) of Maharashtra and were brought to the laboratory in polyethylene bags. Samples were passed through 2mm sieve to remove the larger soil particles and were mixed thoroughly to obtain a composite sample. Later, the composite sample was processed three times to get the mean value. A part of composite soil collected from field was used for physicochemical characterization. Soil was spread out on a tray for air drying. It was sieved over a 2 mm sieve and used for characterization. Soil analysis was done for colour, pH, Electrical Conductivity, Organic carbon %, and major and minor nutrients [Nirogen (kg / hector), Phosphrous (kg / hector), Potassium (kg / hector), Calcium (meq.), Magnesium (meq.), Sodium (meq.), Zinc (ppm), Ferrous (ppm), Mangenese (ppm), Copper (ppm) and Boron (ppm)]. pH of the soil was measured potentiometrically in a 1:5 soil – water suspension by pH meter. Electrical Conductivity (dS/m) which provides concentration of soluble salts in the soil was measured in 1:5 soil-water suspensions by conductivity meter. Organic Carbon was evaluated (14) method by oxidizing organic carbon with potassium dichromate and sulphuric acid. Available Nitrogen was assessed by Kjeldhal tube (16). Available Phosphorus in soil was determined by Olsens method by using spectrophotometer (17,18). Water soluble and exchangeable Potassium was calculated by Ammonium acetate method (19) using Flame photometer. Calcium and Magnesium cations were estimated by EDTA titration (20). Analysis of Ferrous, Mangenese, Copper and Zinc were done by acid digestion of soil (21).

Mycorrhizal Infection

Marsilea quadrifolia roots and rhizomes were collected along with the rhizosphere soil from Naldurg (17.820N 76.30ºE) region of Maharashtra, during 2015-2016 in separate ziplock polythene bags season wise. AM infection was estimated by the techniques (22). For this, the roots and rhizomes were washed in water. Fifty pieces each of rhizomes and roots, each piece 10-mm long, were taken and washed four to five times in fresh water. Then they were boiled in 10% KOH at 90 ºC for 2 hr, again washed in water, acidified in 1% HCL for 3-4 minutes and stained in 0.05% trypan blue. Stained specimens were examined under a stereozoom microscope and observed mycorrhizal colonization, including the presence of arbuscles, vesicles, dark septate endophytes and hyphal, was recorded & calculated % of root colonization. Mycorrhizal spores from rhizosphere soil were isolated by wet and decanting methods (23). For this, three samples, each of 100 g of rhizosphere soil were sieved through a series of wire meshes, having a mesh size 355-25 µm sieves. The residues from sieves were transferred to petri dishes containing distilled water. The number of spores present in the residue was counted using a Binocular microscope (Lawrence and Mayo LM-52-3521). Samples of spores were mounted on slides containing polyvinyl alcohol. The spores were identified using the appropriate manuals (24) & matching original descriptions and those provided by the International Collection of Vesicular Arbuscular Mycorrhizal fungi (http://invam.caf.wvu.edu).m*Marsilea quadrifolia* plants were collected from the natural conditions in monsoon. The same plant was grown on greenhouse for the study of three seasons. In the experiment, sterilized soil were amended with healthy spores was inoculated in the soil surrounding the roots of *M. quadrifolia.* After three months roots and rhizospheric soil were collected and it was checked for AMF spore density and root colonization in the entire three seasons viz. monsoon, winter and summer.

3. Results and Discussion

The experimental findings obtained from the present study have been discussed in the following heads.

Soil Analysis

Soil analysis results revealed that alkaline with pH of 7.71. The pH of the reject was alkaline thus posing no problems for plant growth. Electrical Conductivity (EC) of 0.14 dm/S. Electrical Conductivity (EC), Phosphorus (P) Boron (B) and Sodium (Na) were very poor indicating that there are salinity and plant growth problems. The results revealed that the rejects were more in Organic Carbon (OC), Nitrogen (N), Potassium (K), Calcium (Ca), Magnesium (Mn), and sulphur (S). Zinc (Zn), Ferrous (Fe) and Molybdenum (Mo) is favorable for the plant growth (Table 1). It was studied relation between soil characters and occurrence of AMF where greater number of AM fungal propagules were found in neutral to slightly alkaline (pH 7 to 8) soil where as alkaline soils (pH higher than 8.0) have not favored mycorrhizal fungi (25). AMF have the potential to improve physical, chemical and biological quality of soil by increasing 'C' input in soil (26) and formation and maintenance of soil structure. Organic matter acts as adhesive for binding soil components and improve water infiltration and water holding capacity. Organic carbon or organic matter is the indicator of soil quality and productivity (27), similar observations have been recorded earlier (28).

Mycorrhizal Infection

Among all the three seasons spore density and percentage of root colonization was found to be more in the winter season. Spore density was found more in winter season (425±12.33/100 g soil) and less than monsoon season (03±1.11/100 g soil) followed by summer. Among AMF spore viz. *Sclerocystis,Glomus,* and *Gigaspora* genus was found but *Glomus* found dominant.AMF percentage of root colonization was increased in winter season (35.41±5.11%) while less in monsoon (16.66±2.11%). AMF root colonization types are arbuscular (A), vesicular (V) hyphal (H) and DSE were observed in all season but vesicular and arbuscular types are absent. Winter season showed very significant AMF colonization, spore density and AMF genera (Table1; Plate 1, 2, & 3). Earlier investigators either did not observe endomycorrhiza on aquatic plants or where such observations were made, the frequency of colonization was poor (29). A low colonization rate of 12%–20% in some pteridophytes of Sakleshpur, Karnataka; and 25%–75% in the Nilgiri and Kodaikanal hills of Tamil Nadu, has been recorded (30, 31). Among the pteridophytes of Coimbatore, Tamil Nadu, a wide range of colonization (15%–70%) was reported, but colonization was not observed in *Marsilea minuta* (32). In the present investigation as many as 12 species of VAM fungi were found in the rhizosphere of *M. minuta* of which the spores of three species, *Glomus monosporum*, *G. fasciculatum*, and *A. scrobuculata* were dominant (33). The occurrence of arbuscular mycorrhizas in the

pteridophytes of Yunnan largely agrees with the report (34). The data revealed that the much lower percentage (17%) of arbuscular mycorrhizal plants in the pteridophytes than in angiosperms (62%) (35). It was studied the vesicular-arbuscular mycorrhizas (VAM) of 101 species of pteridophytes in New Zealand (36). The result revealed that all the tested pteridophytic plants (viz. *Equisetum* sp, *Marsilea* sp, *Nephrelepis* sp, and *Adiantum* sp) had AM association in the roots and spore population in the soil. However, maximum root colonization was observed in *Equisetum* spp (96%) where as minimum was observed in *Nephrelepis* spp (28%) (37). Similarly reported the aquatic plant species of *Marsilea* sp were infected 32% root colonization (38). It was observed that the AM fungal such as hyphae and vesicles colonization from the Pteridophytic plant species of an unidentified *Selaginella* sp, and *Adiantum lunulatum* of Selaginellaceae and Adiantaceae family respectively (39). In recent, it was recorded fungal association in gametophytes and young saprophytic roots of *Nephrolepis exaltata* (40).

Table 1: Physico-chemical analysis of rhizospheric soil of *Marsilea* plant.

Sr. No.	Parameters	Value ±
1	pH	7.27 ±1.01
2	Ele. conductivity (d/mS)	0.14 ±0.02
3	Organic carbon (%)	5.93 ±2.02
4	Nitrogen (kg / hector)	370 ±13.12
5	Phosphrous (kg / hetor)	13.78 ±2.11
6	Potassium (kg / hector)	1163.9 ±5.33
7	Calcium (m. Eq.)	6.82 ±1.01
8	Magnesium (m. Eq.)	17.93 ±3.01
9	Sodium (m. Eq.)	0.38 ±0.03
10	Zinc (ppm)	3.64 ±0.22
11	Ferrous (ppm)	3.9 ±0.12
12	Mangenese (ppm)	4.7 ±0.13
13	Copper (ppm)	1.03 ±0.01
14	Boron (mg/gm)	16 ±2.24
15	Sulfur (mg/kg)	7.69±0.11
16	Molybdenum(mg/kg)	1.08 ±0.04

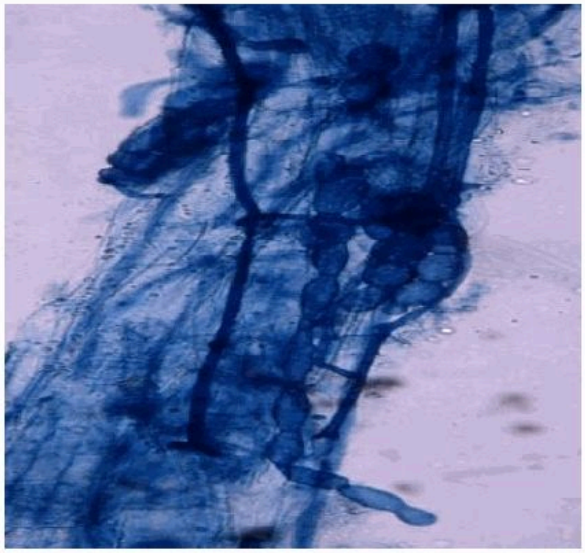
Legends: Values are mean of three readings, Value after ± - Indicates Standard deviation.

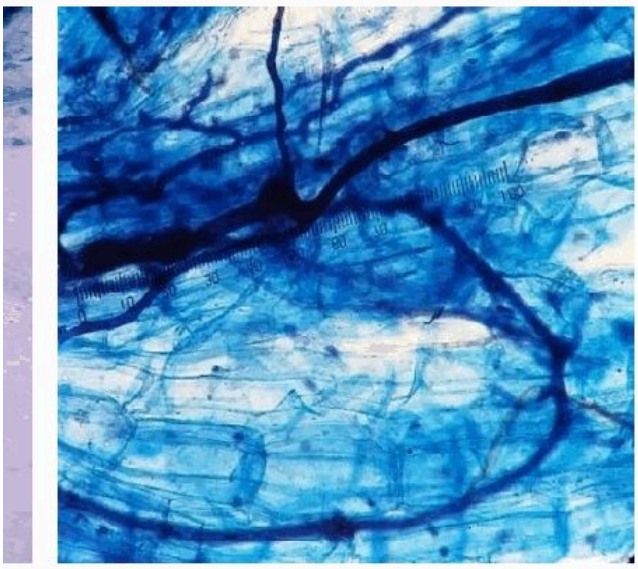
Table 2: Status of Arbuscular Mycorrhizal Fungi (AMF) in *Marsiliea quqdrifolia* season wise.

Sr No	Season	Spore Density/ 100 gm soil	Types of root colonization (%)				Total root colonization (%)	Types of AMF Genera
			V	A	H	DSE		
1	Monsoon	03±1.11	-	-	14.58±1.21	2.08±1.34	16.66±2.11	Acaulospora, Glomus
2	Winter	425±12.33	-	-	31.25±2.44	4.16±0.23	35.41±5.11	Acaulospora, Glomus Sclerocystis
3	Summer	324±15.23	-	-	25±4.22	2.08±0.22	27.08±0.33	Glomus

Legends: Values are mean of five readings. ± - Indicates Standard deviation**,** DSE= Dark Septate Endophytes, H= hyphal, V= vesicular, A= arbuscular.

Plate 1. Type of AMF Colonization (x400).





Swelling (Monsoon) DSE (summer)

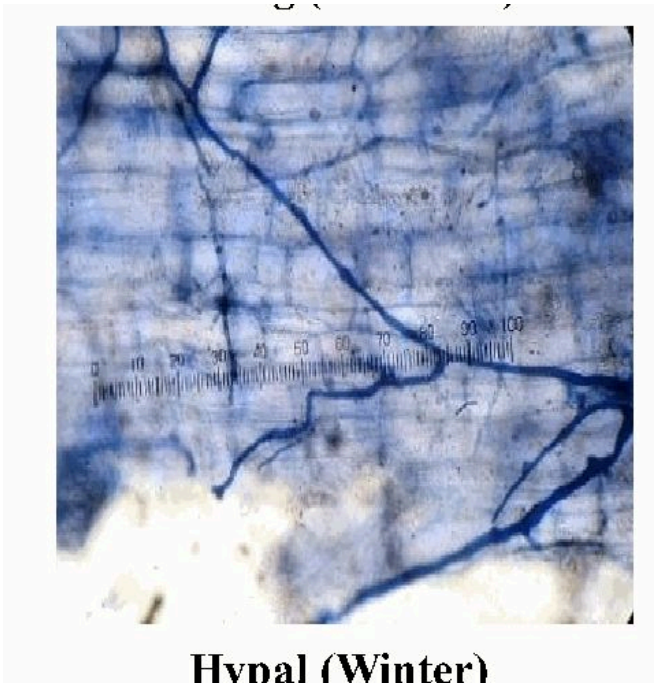
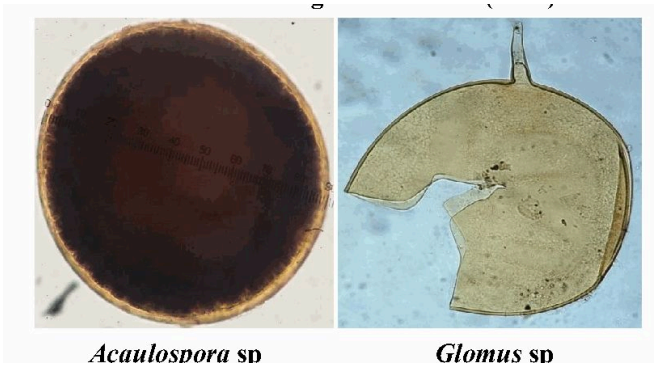
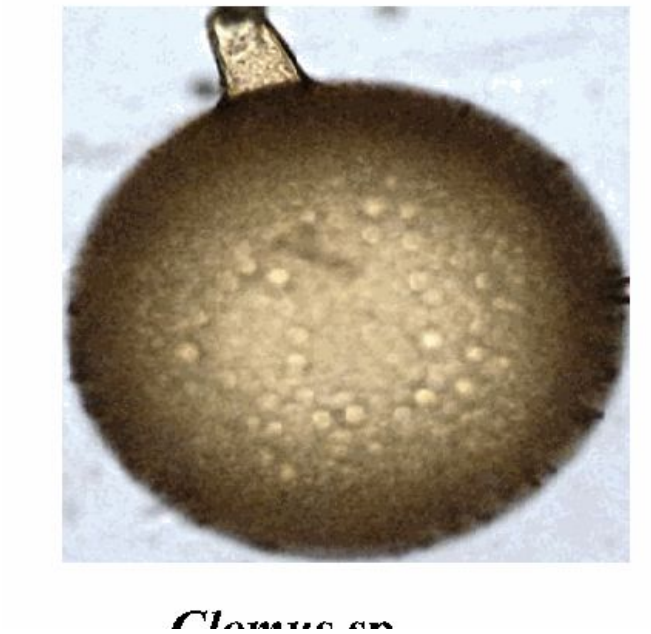


Plate 2. Identified genera of AMF(x400).

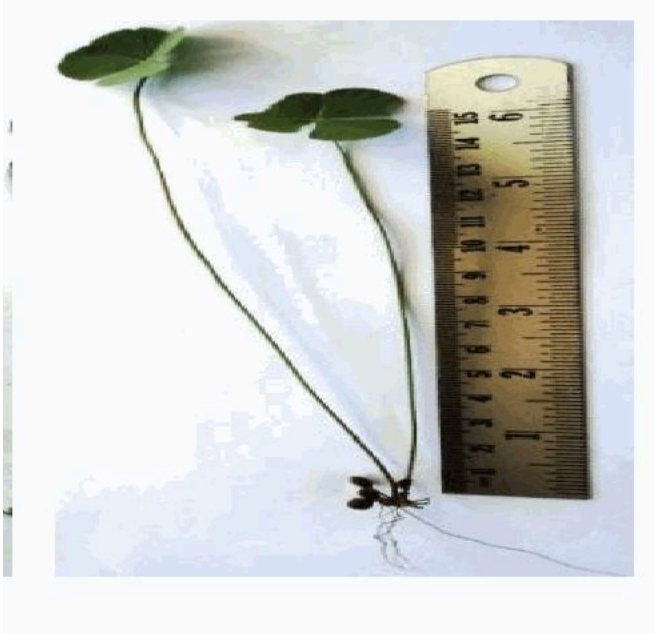




***Glomus* sp *Sclerocystis* sp**

Plate 3. *Marsilea quadrifolia* habit.





4. Conclusion

It was concluded that soil factors indicating plant growth problems. Percentage of Arbuscular mycorrhizal fungal (AMF) infection, number of resting spores and genera varied for all the three seasons. Among all the three seasons spore density and percentage of root colonization was found more in the winter season. It is believed that the AM fungi may be playing some role in nutrient absorption, especially in plants growing in nutritionally poor conditions in some extent. Perhaps we should consider AM to be a long-term strategy that incorporates the flexibility to survive with both present and future environmental conditions. Thus, further need to be identification and isolation of dominant native AM fungal species successful on pteridophytes (*Marsilea qudrifolia)* plant species, their multiplication and proper utilization would make the re-establishment and regeneration attempts ecologically and economically viable in such constrained agricultural ecosystems.

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