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UNDERSTANDING THE ROLE OF FUNGAL DIVERSITY ASSOCIATED WITH CARNIVOROUS PLANT ROOT

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ABSTRACT

Carnivorous plants grow in low soil and low nutrient concentration. They depend on the insect for nitrogen source and also perform photosynthesis to obtain energy. However, how do they trap the nutrients from soil is debatable. To uncover this fact, we intend to study the fungal association in the rhizosphere of *Utricularia purpurascens* and *Utricularia striatula* obtained from Amboli. The fungal association was considered because the root systems of carnivorous plants are weakly developed. Based on the understanding of fungal structures like the presence of hyphae and mycelia, fungi may aid in nutrient acquisition for the plant. Like most plants face a trade-off with growth, reproduction, and development, they evolve to produce some volatile components or signaling molecules. To understand this mechanism, rhizosphere soil of the carnivorous plants was diluted. The soil around the roots of the plant was used to culture the fungus and to perform the morphological identification. Ten different fungi were observed in the culture plate. These fungi were further checked for pectinase, chitinase and protease activity. These activities are thought to up-regulated in response to insect or worm prey observed around the rhizosphere. Indole acetic acid production, phosphate Solubalization activities were also considered to comprehend nutrient absorption by the plant. Since little is known about the associations with carnivorous plants and fungi, this study may give an insight into rhizosphere interaction with Carnivorous plants. It may also aid to understand the trapping strategy utilized by prey and predator in search of nutrients.

Key Words : Carnivorous plants, *Utricularia*, Fungal association, Rhizosphere, Phosphate solubalization

INTRODUCTION

Soil acts as a home for different organisms, a large part constitute the microbes. These microbes plays critical role in decomposition, nutrient acquisition and in nutrient cycling. As per the soil type there is change in microbial diversity. One of these type is Fungi, acquires their food by absorbing dissolved molecules, typically by secreting digestive enzymes into the environment. Fungus is present in soil as well as in association with plant and their roots. Such mycorrhizal, arbuscular and endophytic insect pathogenic fungal associations are one of the adapted mechanisms by roots for utilizing nutrients from soil which are as studied by Behie.[1] According to Quilliam, fungi are associated with roots of carnivorous plants. Carnivorous plants are known for their special mechanisms for nutrition uptake by capturing insects.[2,3] For example *Drosera, captures* insects to fulfill its nutrient requirement as they grow in soil with low nitrogen, phosphorous and potassium content. Great diversity of insectivorous plants were studied by kumar in India, his study mainly focuses on insectivorous plants and the habitat they are found like *Drosera* species found in crop lands, plains near water bodies, the *Pinguicula* species found in Himalayas, *Nephenthes* found in North-Eastern part of India, *Utricularia* found in and around the water bodies.[4] These plants have different Prey trapping systems

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both by the phyllosphere and the rhizosphere. The bacterial system active in the flower is well documented.[5, 6] Very scarce reports are available on nutrient uptake by the roots. Different insectivorous plants has different roots types as well like tuberous, fibrous, vertical stolon attach to rocks, single ephemeral in Genera *Pinguicula* and some Genera like *Utricularia* are rootless [2]. Carnivorous plants have very tiny roots, making the nutrient uptake difficult. We hypothesize that does fungal associations aids the plant in nutrient acquisition for the plant. The morphology of the fungi may help in the nutrient acquisition by spreading its mycelia in large area of soil and transfer soil nutrients to the plants by secreting some extra or endo-cellular enzymes to breakdown complex food substances such as protease and by converting inorganic components to organic form. It is also found out that some insect pathogenic fungi kills the insect and transfer its nutrients to plant.[1,7] Thus it becomes interesting to study the carnivorous plant-fungal associations. This interesting association may help to uncover many facts.

Hypothesis : Fungal association with insectivo-rous plants roots helping in transferring the nutrients to the plant

AIMS AND OBJECTIVES

1. Exploring fungal diversity associated with the insectivorous plants.
2. To study the role of fungus associated with the roots of the plants.

MATERIAL AND METHODS

1. **Plant sampling and collection :** The carnivorous plants namely *Utricularia purpurascens* and *Utricularia striatula* were collected from region of Amboli, in the month of August, and October, 2019. The soil around the root was also collected in autoclaved containers and stored in -20°C till further use.
2. **Culturing the fungus and microscopic observation :** We have used a culture dependant method to understand the fungi around the root. The soil sample around the plant root serially diluted and 10^{-3} dilution was use for diversity studies. The dilution was plated on Potato dextrose agar with 50µg of Chloramphenicol to avoid the growth of bacteria. Growth of fungi was observed after 7-8 days. The microscopic observations were done using a Binocular microscope (Lawrence Mayo, Model no: LM-52-1710). The fungal morphology observed and studied for identification of the fungi.**(Fig. 1)**
3. **Biochemical assays:** To understand if there are any fungi associated with the roots of the carnivorous plant that pass on the food to the plant, the biochemical tests were performed. The tests were selected on the basis that will help the plant to receive the nutrients from the soil. Pectinase[8], protease[9], chitinase[10], phosphate solubalization[11] and indole acetic acid production[12] were tested in the observed fungi.

RESULTS AND DISCUSSION

Fungal diversity associated with carnivorous plant

On the basis of morphological and microscopic observations a great diversity of fungi were obtained from 10^{3} dilution plates of the rhizosphere soil.**(Table 1)** Out of 11 fungal isolates, total of 7 fungi isolates were successfully identified from *Utricularia striatula* and *Utricularia purpurascens.* However, due to large number of fungal isolates, they were determined on the basis of morphological characteristics (colony form, colony color, colony margin, spore arrangement and pigment production). The isolates were classified into different groups according to their genus that are *Trichoderma, Aspergillus, Fusarium, Mucor, Penicillium sp. The dominating genus in Utricularia striatula plant was Aspergillus spp and in Utricularia purpurascens was Penicillium sp.*

Table 1 : Morphological characteristics of identified fungus

S/N	Fungal genus	Fungal SPECIES	Associated plant	Color
1	*Trichoderma*	-	*Utricularia striatula*	Green
2	*Aspergillus*	*Aspergillus niger*	*Utricularia striatula*	Black
		Aspergillus terreus		Brownish
		Aspergillus flavus		Greenish yellow
3	*Fusarium*	-	*Utricularia striatula*	Purple-White
4	*Mucor*	-	*Utricularia striatula*	Off White
5	*Penicillium*	-	*Utricularia purpurascens*	Greyish

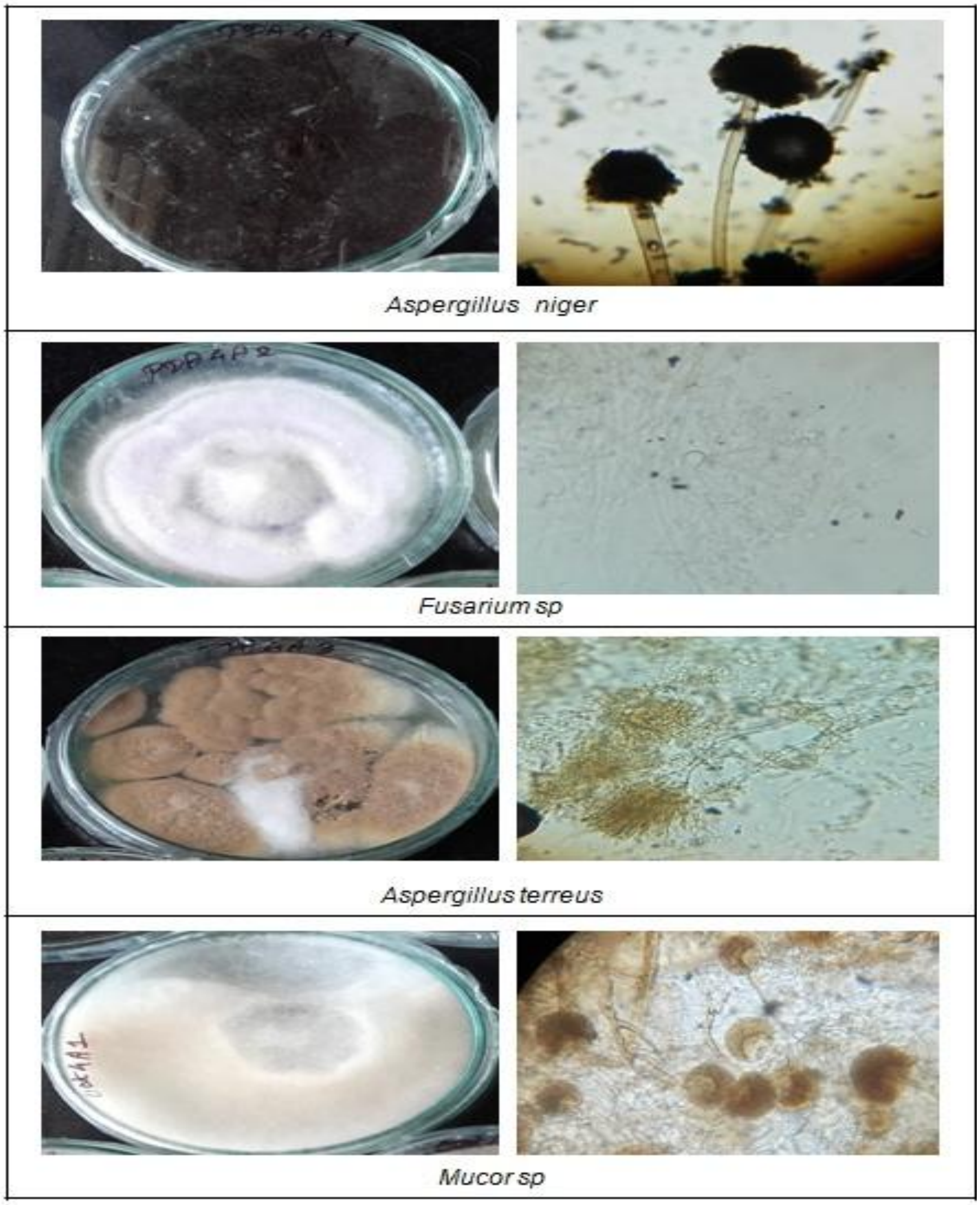

Fig. 1 : Microscopic observations of identified fungus and its pure culture plates

Bio-chemical Assays

Protease assay: To check whether the fungus has the ability to produce extracellular enzymes this helps in protein hydrolysis of small polypeptides and amino acids in nitrogen metabolism.[13-15] Hence this test was performed using cultured protease agar plates which was flooded on 8th day with1M HCL. The fungi were not able to utilize protease present in media.[16-18]

Pectinase assay: To check whether the fungus degrades a complex pectin polysaccharide.[15] As this degradation process show one way of invading the host tissue. This assay was conducted using cultured Pectinase agar plate which was flooded on 8th day with 4.53% cetrimide. No clear zone around the colony indicates pectin was not degraded.[19-20]

Chitinase test: Understanding the activity of fungi involve in chitin degradation by hydrolyzing the glycosidic bonds of chitin which after degradation acts as carbon source in carbon and nitrogen cycle. No fungal growth on the chitinase agar plate indicates the fungi were not utilizing the colloidal chitin present in the medium.

Phosphate solubilization test : Phosphate solubilization test was carried out to check the phosphate dissolution indicating conversion of inorganic phosphate to organic form by the fungi.[21] This test showed positive results as *Aspergillus terrus* was observed on the plates which showed zone of clearance. This result helps in understanding that the fungus is utilizing phosphate. Solubilization efficiency (SE) was calculated using diameter of clear zone (4mm) by the diameter of colony (20 mm) which is found to be SE unit 0.2.

Indole acetic acid test : IAA was performed to check production of IAA which is a plant growth promoter hormone. For IAA the fungal samples were taken to check for the O.D. at 525nm and 545nm and its mean concentration was plotted on graph with the standard IAA for comparison **(Fig. 2)**. The concentrations of samples were found by formula:

$$\text{Unknown Concentration} = \frac{\text{Test O.D.}}{\text{Standard O.D}} \text{ X Known Standard Concentration}$$

The graph represents that IAA was found to be maximum of *Trichoderma sp* followed by *Aspergillus flavus, Penicillium sp and Mucor sp*.

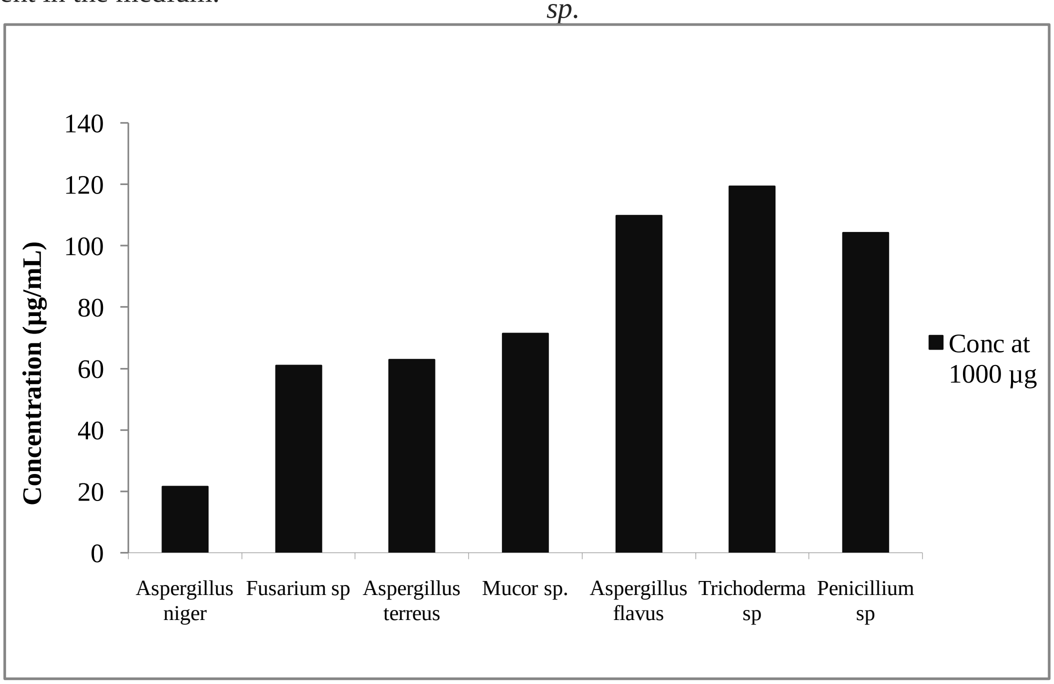

Fig. 2 : Concentration of IAA produced by fungi found around the roots of carnivorous Plant

The soil around the Carnivorous plant root has a great diversity of fungi which may be involved in nutrient transfer to the plant needs further tests. From the observed result of pectinase and protease test it was interpreted that if there is no clear zone around the colony then the colony is not able to break done the nutrients present in the medium. Phosphates solubilization test was found to be positive by 1 fungi colony that is *Aspergillus terrus* states that the fungi is able to breakdown the phosphate present in the medium and utilize it as a source of nutrients resulted in zone of clearance around the colony. In Chitinase test the fungi is not able to utilize the colloidal chitin present in the medium and thus is not able to grow on the plate. From **Fig. 2** it was observed that the maximum concentration of IAA was produced by *Trichoderma sp* (119.24µg) that help in promoting growth of plant followed by IAA produced by *Aspergillus flavus*, and *Penicillium sp*. however *Aspergillus niger* produced the least concentration IAA.

CONCLUSION

Diversity of fungi were observed around the roots of carnivorous plants which helps in nutrient transfer by breaking the complex substances and utilizing them from soil. Maximum Concentration of IAA was by *Trichoderma sp*. Indicating the role of fungi in nutrient transfer to the plant. Few more assays may aid in understanding the role of fungi in nutrient transfer.

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Conflict of interest : The authors declare no conflict of interest in the present study.

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