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# Diversity of Rhizosphere Fungi and Soil Nutrient Properties of *Amorphophallus sylvaticus* (Roxb.) Kunth

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**Abstract:** ***In present investigation diversity of rhizosphere fungi of Amorphophallus sylvaticus (Roxb.) kunth a monsoon perennial cormatus plant species belonging to family Araceae was studied from two sites of Nanded district by using serial dilution and soil plate methods on the Czapek's Dox Agar and MRBS Agar medium. Soil chemical properties, including pH, electrical conductivity, organic carbon, P, K, Cu, Fe, Mn, and Zn content, were also analyzed. The soil nutrition status shows low content of macronutrient and it may be negatively influence on rhizosphere fungal diversity of plant. Quantitative analysis of fungal population of Pota site is greater than Nageli site. A total 21 fungal species from rhizosphere of Pota site and 16 species from Nageli site were isolated and identified. The rhizosphere fungal population of each site was correlated with the soil chemical properties of each site and there is a significant correlation between fungal populations with the chemical properties investigated. The most dominant species includes Aspergillus flavus, Aspergillus niger, Penicillium chrysogenum, Penicillium citrinum, Rhizopus stolonifer, Rhizoctonia sp., Fusarium oxysporum, Trichoderma harzianum & Alternaria alternata.***

**Keywords:** Rhizosphere fungi, Diversity, *Amorphophallus sylvaticus* (Roxb.) Kunth and Soil nutrients

## 1. Introduction

*Amorphophallus sylvaticus* (Roxb.) Kunth is a monsoon perennial cormatus plant growing in forest and bands of cultivated fields of Marathwada region in Maharashtra (Naik, 1998). It produces inflorescence and flower during month of May. Rhizosphere is a zone around the plant roots which shows a huge diversity among the microbes. A comparison of the numbers of known and estimated total species of microorganisms in the world indicates that 95% of fungi, 78% of bacteria and 96% of viruses still remain to be discovered (Bull *et al.*, 1992). Many workers have reported that greater numbers of microorganisms are present in the rhizosphere soil than in the non-rhizosphere soil. (Ames, 2000; El-Amin & Saadabi; 2007). One of the most fascinating hot spots of activity and diversity in soils is the rhizosphere (Jones and Hinsinger, 2008).

Studies revealed that Fungi are an important component of the soil microbiota typically constituting more of the soil biomass than bacteria, depending on soil depth and nutrient conditions (Ainsworth and Bishy, 1995). One of the most important factors responsible for the growth of microorganisms is organic substances exuded by roots i.e. root exudates (Liljeroth and Baath, 1988). The exudates include simple sugars, amino acids, organic acids, vitamins and many other compounds (Singleton and Sainsbury, 1991; Klein, 1992). Soil physico-chemical characteristics also have a great impact on microbial biomass and microbial activity and can be used to measure soil quality (Parr and Papendick, 1997).

Many studies showed that the physiological activities of the rhizosphere microorganisms had an important influence on soil properties, nutrient uptake and plant growth and development (He and Li, 1999). The area of the soil influenced by root varies with the type of plant, age of the plant, soil conditions, and pH of the soil, environmental conditions and moisture content of the soil which alter qualitative as well as quantitative distribution of fungi in the rhizosphere and non-rhizosphere soil. (Harley & Waid, 1955; Burges & Raw, 1967).

There are plenty reports on rhizosphere fungal diversity of different plants like *Aesculus indica* (Anand and Rupinder, 2010), *Ceropegia bulbosa* (Mulani and Turukmane, 2014), species of Myristicaceae (Rama Bhat & Kaveriappa, 2011), Chilli field (Gomathi *et al.*, 2011), *Aloe vera*, *Argemone maxicana*, *Abutilon indicum*, *Amaranthus polygamus* and *Achyranthus aspera* (Srivastava and kumar, 2013). But there is a no of report on rhizosphere fungal diversity of *Amorphophallus sylvaticus* (Roxb.) Kunth was available so far.

The purpose of present investigation is to study rhizosphere fungal diversity and its correlation with soil chemical properties of *Amorphophallus sylvaticus* (Roxb.) Kunth from two different sites.

## 2. Materials and Methods

### Collection of Rhizosphere Soil Samples

Rhizosphere soil samples were collected from *Amorphophallus sylvaticus* (Roxb.) Kunth fields of village Pota, Tq. Himayatnagar and Nageli, Tq. Mudkhed of Nanded district during winter (November, 2014) by digging out soil around the rhizosphere area up to 20 cm from plant to a dimension of 15 cm height and 7 cm diameter. The three soil samples were collected from each sampling site and mixed together into a single. These soil samples were collected in sterile polythene bags and brought to the laboratory.

**Analysis of Chemical Characteristics of soil**
The different soil parameters were analyzed by using different methods such as pH & Electrical conductivity (Agriculture dept. of Maharashtra), Organic carbon (Walkey and Black method 1934), Phosphorus (Olsen's Method 1965), Potassium (Hanway and Heidel 1952), Copper, Iron, Manganese and Zinc (Lindsay & hornvell Method ) were conducted Rashtriy chemical and fertilizers soil testing lab Nanded.

**Analysis of Fungal Diversity**
The rhizosphere fungi were enumerated by two methods such as Serial dilution (Waksman, 1922) & soil plate method (Warcup, 1950). Dilution of $10^{-2,}$ $10^{-3}$, $10^{-4}$, and $10^{-5}$ were used to isolate fungi on MRBSA and Czapek's Dox Agar. In soil plate method 0.005 to 0.15 g (approximately) air dried soil transferred into the 6 sterile petriplates with the help of a sterilized cooled loop or transfer needle. One percent streptomycin solution was added to the medium before pouring into petriplates for preventing bacterial growth and plates were kept for incubation at 28 ºC for 4-7 days for fungi.

**Observation and Identification**

The individual colonies of fungi were selected based on morphology and purified by inoculation on Czapek's- Dox & PDA agar plates which were incubated for 7–14 days at 28ºC. Then After 7-14 days, the selected colonies were counted and isolated from different plates and transferred to agar slants. The slants were incubated at 28$^{o}$C for 7 to 10 days.

The fungal morphology were studied macroscopically by observing colony features (Colour and Texture) and microscopically by staining with lacto phenol cotton blue and observe under compound microscope for the conidia, conidiophores and arrangement of spores . The microphotograph was taken for isolated species by using Magnus camera. The fungi were identified with the help of literature identification of the species (Barnett and Bary, 1998, Gilman, 2001, Nagamani *et al.,* 2006).

**Statistical analysis:-**

The quantitative analysis of fungal population was studied at $10^{-3}$ dilution. The percentage contribution of each colony forming units (CFU) of different fungal isolate was calculated by using the formula.

$$\text{CFU/ g dry soil} = \frac{\text{Mean plate count X dilution factor}}{\text{dry weight of soil}}$$

$$\text{Percentage contribution} = \frac{\text{Total no. of CFU of an individual species X 100}}{\text{Total no. of CFU of all species}}$$

## 3. Results & Discussion

The soil analysis result shows the pH and Ec were higher in Site B while Cu and Mn were more in site A. Organic carbon, potassium, Fe and Zn were low and phosphorous content is very low in both site in point of view of soil fertility. Except pH & Ec the all analyzed soil chemical parameters from site A (Pota) were higher than site B (Nageli) (Table.1 & Fig.1).

During this investigation a total 75 colonies from site A (Pota) and 58 colonies from site B (Nageli) were isolated during the Month of November 2014 (Table.3 & Fig.2). The Rhizosphere fungal populations at $10^{-3}$ dilution were vary in both site and it is maximum in site A (Pota) compared to site B (Nageli) (Table.2).

A total 21 and 16 fungal species were isolated and identified from site A (Pota) & site B (Nageli) respectively. The most dominant species are *A. flavus, A. niger, Penicillium chrysogenum, P. citrinum, Alternaria alternata, Trichoderma harzianum* and *Rhizopus stolonifer*. The total fungal species in site A (Pota) were higher than site B (Nageli).The percentage contribution of fungal species is varying in both sites. A 5 different species namely are *Aspergillus terrus, Fusarium moniliformae, Mucor sp., Rhizoctonia & Trichoderma viride* only found in site A (Pota) while 16 species were common for both site (Table.3 & Fig.3).

The different research study on diversity and distribution of different fungal species revealed that the diversity of fungi largely depends upon physico-chemical parameters of soil.Various researches revealed that the variations in fungal diversity in some soil types were due to changes in soil organic contents, pH, water holding capacity and temperature of respective season (Dkhar & Mishra, 1987).

The fungal populations were correlated with nitrogen levels and soil moisture (Lorgio *et al*., 1999) and they were statistically significant. The abundance of microorganisms in soil varies spatially as well as temporarily, and this pattern is related to temporal and spatial variations in the quantity and quality of nutrients (Nedwell and Gray, 1987). Fungal diversity of any soil depends on a large number of factors of the soil such as pH, organic content and moisture (Rangaswami *et al.*,1998).

The Soil pH, organic content and water are the main factors affecting the fungal population and diversity (Yu C *et al.,* 2007).The organic carbon, nitrogen, phosphorus, potassium are important nutrients which affect the growth of fungi. In the absence of any of these the growth and sporulation of moulds as well as other microorganisms are hampered a lot (Saksena, 1955).

The maximum fungal diversity was found during winter (November) is investigated in sites A (Pota) than site B (Nageli), similar observations were reported at pal forest soil (Rane and Gandhe, 2006). Various research finding revealed that the species of genus *Aspergillus* and *Penicillium* were dominant in soil (Rama Bhat & Kaveriappa, 2011; Gomathi *et al.,* 2011; Shiny *et al.,* 2013; Gopal & Kurien, 2013).

## 4. Conclusion

The present investigation reports the rhizosphere fungal diversity of *Amorphophallus sylvaticus* (Roxb.) Kunth. The diversity and distribution fungi it is correlated with physico-chemical properties. Because there is increasing total

colonies and total fungal species in Site A (Pota) as compared with site B (Nageli). The soil of site B is deficient in chemical properties as compared to site A which influence on the rhizosphere diversity that's the reason for low fungal diversity in Nageli site. From these experimental results we conclude that low nutrient status of soil results in decreasing rhizosphere fungal population and species diversity of plant. It means that there is correlation between fungal population and soil nutrients.

## 5. Acknowledgement

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**Table 1:** Analysis of soil chemical properties of *Amorphophallus sylvaticus* (Roxb.) Kunth

| Sr. No | Parameters | Site A (Pota) | Site B (Nageli) |
|---|---|---|---|
| 1 | pH | 7.4 | 7.6 |
| 2 | Ec (m-mhos/cm) | 0.19 | 0.24 |
| 3 | Organic Carbon % | 0.30 | 0.23 |
| 4 | Phosphorus Kg/h | 2.39 | 1.86 |
| 5 | Potassium Kg/h | 134 | 112 |
| 6 | Copper (ppm) | 2.29 | 1.74 |
| 7 | Iron (ppm) | 1.70 | 1.35 |
| 8 | Manganese (ppm) | 6.09 | 4.21 |
| 9 | Zinc (ppm) | 0.58 | 0.32 |

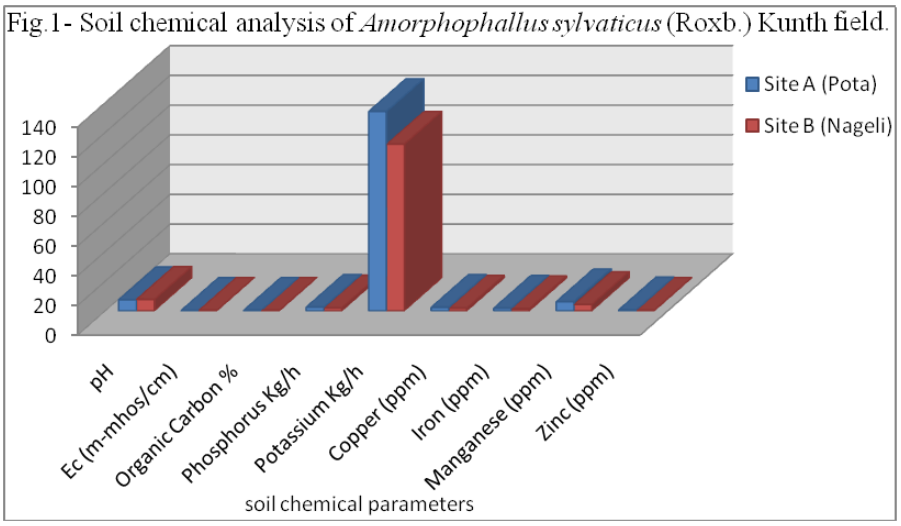
**Table 2:** Rhizosphere fungal population (x $10^{-3}$cfu/g soil dilution) of *Amorphophallus sylvaticus* (Roxb.) Kunth

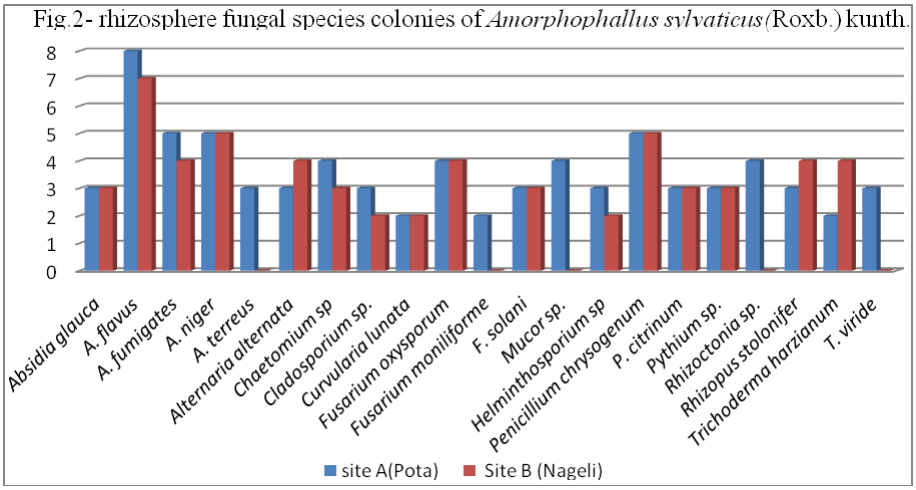
| Sr. No. | Plate No. | Fungal population | |
|---|---|---|---|
| | | Site A (Pota) | Site B (Nageli) |
| 1 | i | 41 | 32 |
| 2 | ii | 42 | 28 |
| 3 | iii | 37 | 25 |
| | Mean | 40 | 28.33333333 |
| | S.D | 40±2.16 | 28.33±2.86 |

**Table 3:-** Total number of colonies and percentage contribution of fungi recorded from Rhizosphere of *Amorphophallus sylvaticus (*Roxb.) Kunth from site –A (Pota) and B (Nageli).

| Sr. No | Site | Site A | | Site B | |
|---|---|---|---|---|---|
| | Name of the fungal species | Total colonies | % contribution | Total colonies | % contribution |
| 1 | *Absidia glauca* Hagem. | 03 | 4.00 | 03 | 5.17 |
| 2 | *A. flavus* Link. | 08 | 10.66 | 07 | 12.06 |
| 3 | *A. fumigatus* Raper & Fennell. | 05 | 6.66 | 04 | 6.89 |
| 4 | *A. niger* Van Tighem. | 05 | 6.66 | 05 | 8.62 |
| 5 | *A. terreus* Thom. | 03 | 4.00 | -- | -- |
| 6 | *Alternaria alternata* (Fr.) Kaissler. | 03 | 4.00 | 04 | 6.89 |
| 7 | *Chaetomium* sp | 04 | 5.33 | 03 | 5.17 |
| 8 | *Cladosporium sp.* | 03 | 4.00 | 02 | 3.44 |
| 9 | *Curvularia lunata* (Wakker) Boedijn. | 02 | 2.66 | 02 | 3.44 |
| 10 | *Fusarium oxysporum* Schltdl. | 04 | 5.33 | 04 | 6.89 |
| 11 | *Fusarium moniliforme* J. Sheld. | 02 | 2.66 | -- | -- |
| 12 | *F. solani* (Mart.) Sacc. | 03 | 4.00 | 03 | 5.17 |
| 13 | *Mucor sp.* Fresenius | 04 | 5.33 | -- | -- |
| 14 | *Helminthosporium sp.* | 03 | 4.00 | 02 | 3.44 |
| 15 | *Penicillium chrysogenum* Thom | 05 | 6.66 | 05 | 8.62 |
| 16 | *P. citrinum* Thom | 03 | 4.00 | 03 | 5.17 |
| 17 | *Pythium sp.*Pringsheim | 03 | 4.00 | 03 | 5.17 |
| 18 | *Rhizoctonia sp.*Kuhn | 04 | 5.33 | -- | -- |
| 19 | *Rhizopus stolonifer* (Ehrnberg) Vuillemin | 03 | 4.00 | 04 | 6.89 |
| 20 | *Trichoderma harzianum* Rifai | 02 | 2.66 | 04 | 6.89 |
| 21 | *T. viride* Pers.ex.Fries | 03 | 4.00 | -- | -- |
| | Total colonies | 75 | | 58 | |
| | No. of Species | 21 | | 16 | |

Total colonies ($10^{-2,}$ $10^{-3}$ & $10^{-4}$) dilution.



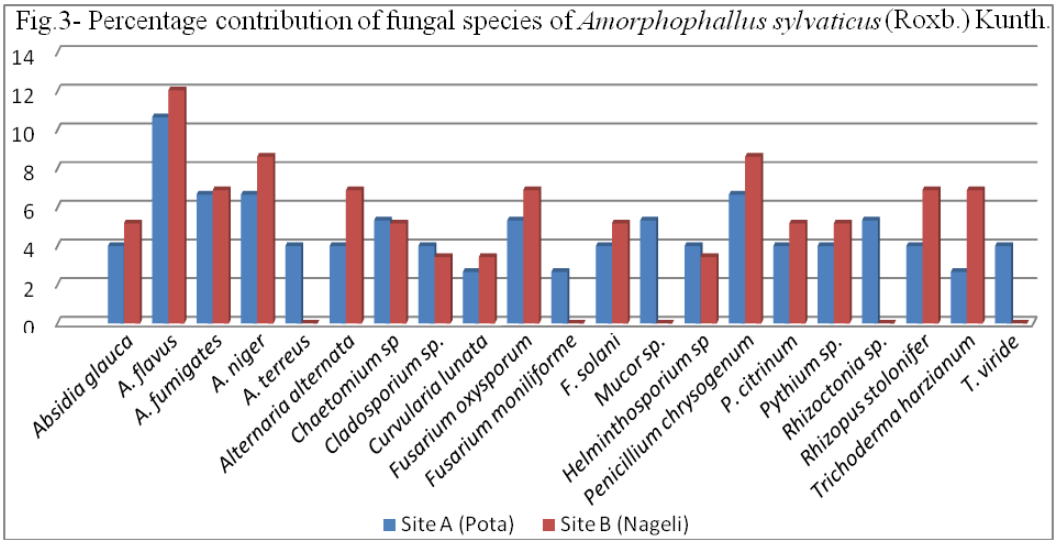
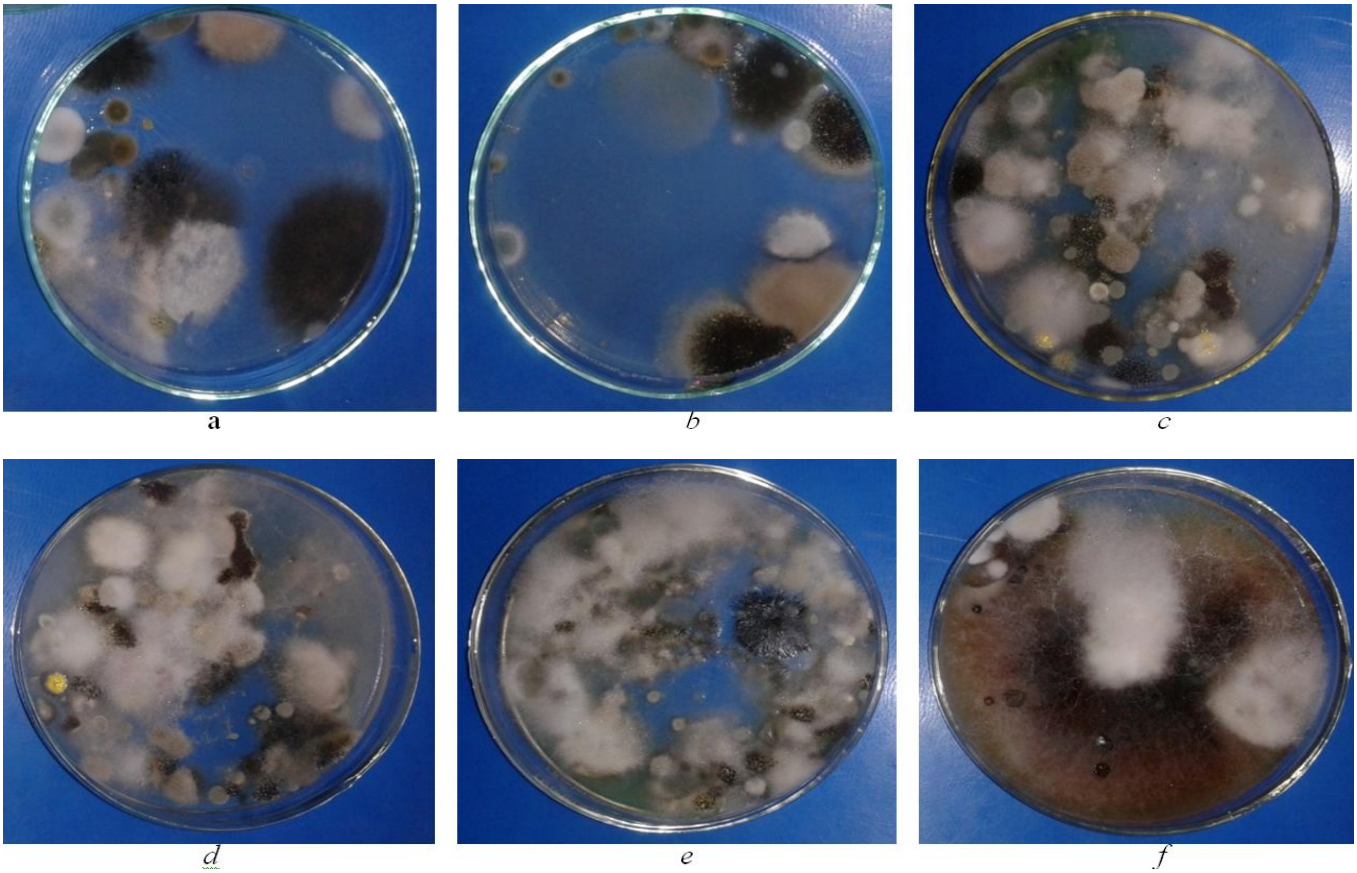




**Plate I (a)-Habit of *Amorphophallus sylvaticus* (Roxb.) Kunth.**

**Plate I (b)** - **Corms and fruting of Amorphophallus *sylvaticus* (Roxb.) Kunth.**

**Plate II** - **Soil Dilution Plate of Rhizosphere Fungi of *Amorphophallus Sylvaticus* (Roxb.) Kunth.**



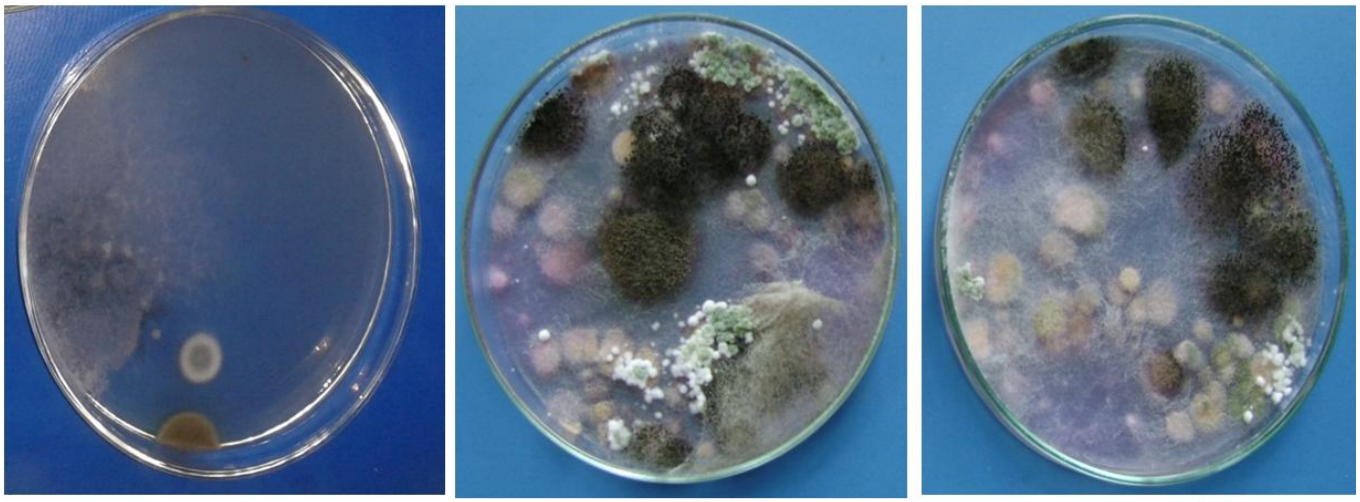


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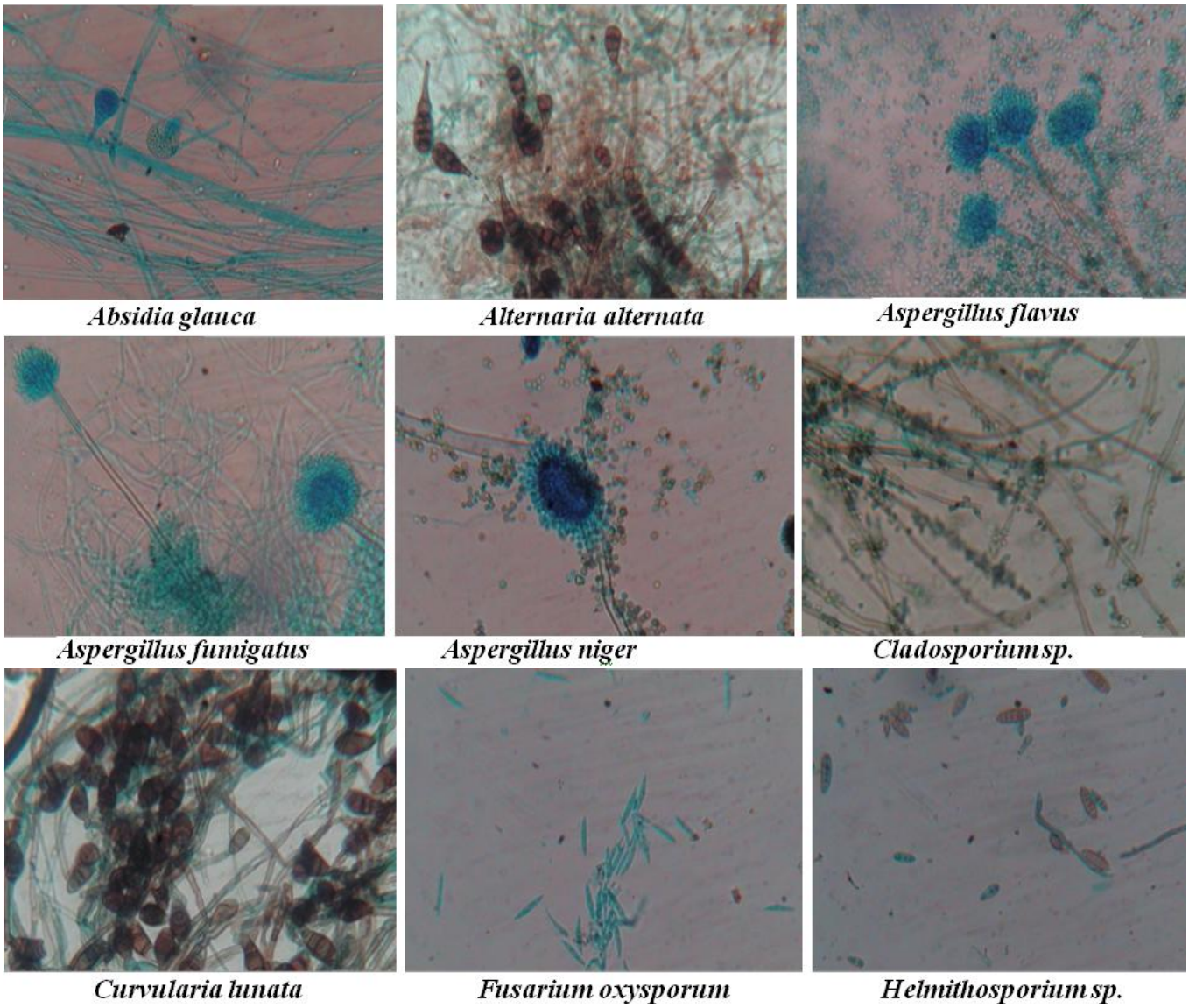
**Plate III-** **Microphotograph of Rhizosphere Fungi of *Amorphophallus Sylvaticus* (Roxb.)**



*Absidia glauca* *Alternaria alternata* *Aspergillus flavus*

*Aspergillus fumigatus* *Aspergillus niger* *Cladosporium sp.*

*Curvularia lunata* *Fusarium oxysporum* *Helmithosporium sp.*

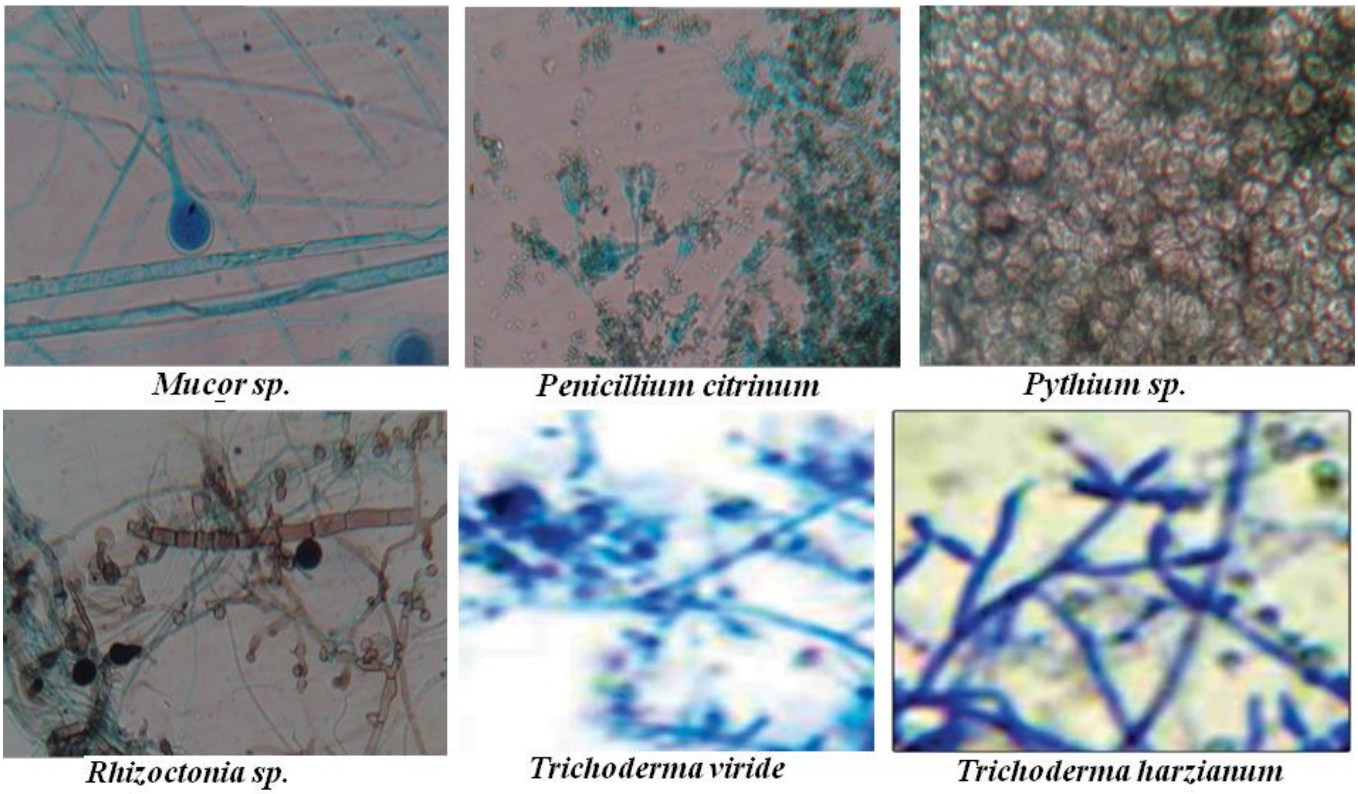
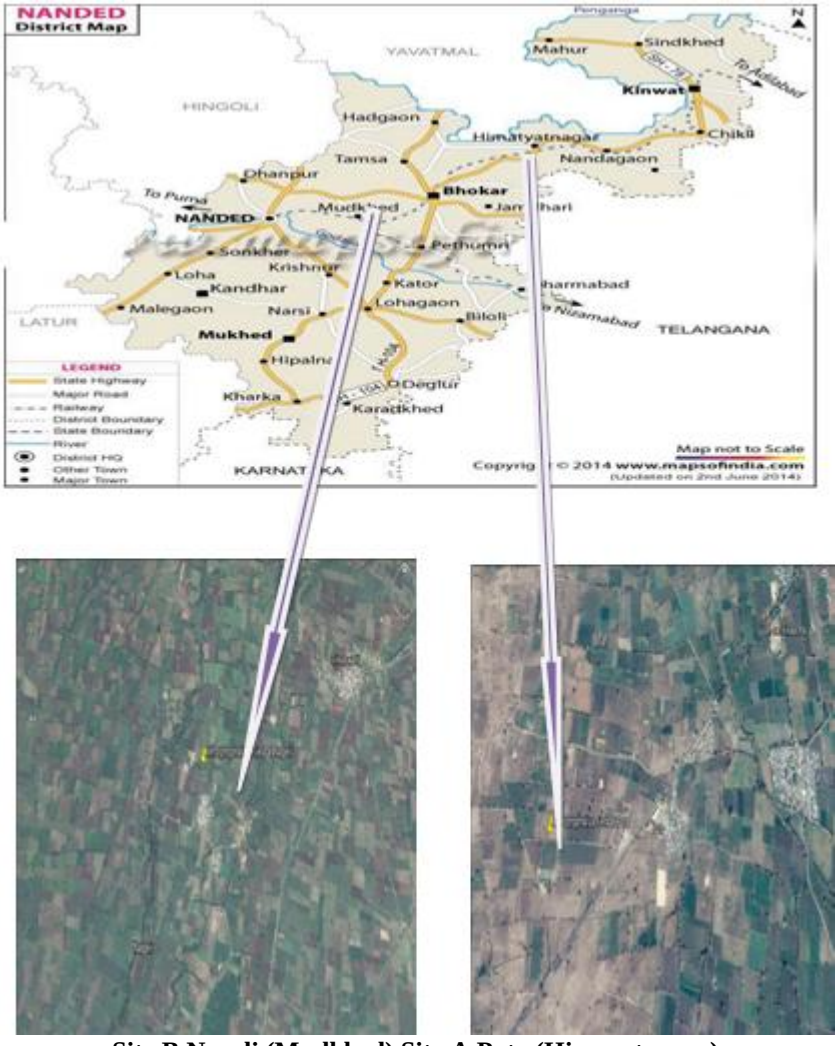



**Plate IV – Field map of *Amorphophallus sylvaticus* (Roxb.) Kunth in Nanded District**




**Site B Nageli (Mudkhed) Site A Pota (Himayatnagar)**

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