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ISOLATION AND IDENTIFICATION OF SOIL MYCOFLORA IN DIFFERENT AGRICULTURE FIELDS OF TILDA, RAIPUR (C.G.)

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ABSTRACT Soil is an essential element for life as they provide a medium for the growth of plants, habitants for many insects and various other organisms as well. Soil also acts as a system of filtration for surface water, storage of carbon related compounds and maintains the atmospheric gases. In the present investigation, 38 fungal species representing 15 genera were isolated and identified from different agriculture fields of Tida. The dominant genera in selected field were *Aspergillus, Penicillium* and *Trichoderma* species. The physico chemical characteristics of soil samples showed variations collected from different months. The present study clearly revealed that the physico chemical parameters influenced the diversity and distribution of soil fungi in agricultural field.

KEYWORDS : Isolation, Soil Fungi, *Aspergillus, Penicillium.*

Introduction

The essential nutrients as well as minerals are provided to the plants through soil. The major role is played by the microorganisms for sustaining the ecosystem of soil. The awareness related to environmental measures has increases rapidly and has resulted into the regulatory measures aiming to provide a remedy to the past mistakes and environmental protection from any possible contamination as well as exploitation. The measures as discussed basically intends on preserving the environment along with human health protection. The use of pesticides is incorporated for the betterment of the crop production but also has limitations and in fact, were banned when it was found that some of the pollutants which are of major concern were actually the resultant of pesticides hazardous to the health of humans. India, a major country of pesticides usage amongst the South Asian countries where majority of the consumption of total pesticides is by cotton crop (Agnihotri, 1999). As a result, it leads to a rapid increase in soil and aquatic environment eventually resulting into crop waste accumulation (Hernandez *et al.,* 2006). The plant growth is sometimes limited due to the growth of fundi in the soil. Most of the times, fungi either does not allow the plant to grow actively or gives a slow metabolization to utilizing a wide range of organic molecules. This is done by distributing the organic matter away from plant roots. It has been shown by a genetic study that in contrast to the plants, fungi are closely related to animals. (Dick, 2009) inferred that fungi possesses 80 or more percentage of the same genes as of humans. Apart from the plant related affects, fungi also play an important role in day to day lives of the humans. However, fungi also provide utility to the agricultural practices, medicine industries, food industries, textiles, natural cycling as well as bio-fertilizer industries. The biotechnology related to fungi has become an integral part of welfare of human beings. A continuous usage of fertilizers over an elongated period of results into an imbalance of soil mycoflora indirectly affecting the biological properties of soil resulting into its degradation (Manickam *et al.,* 1972). Fungus benefits many plants and crops by suppressing the plant root diseases, and by promoting healthier plants attacking with fungal enzymes to others. There exists some microorganism which survives in pesticide contaminated locations as well. Pesticides affects microbial populations of soil and stimulates the growth of certain microorganisms eventually exerting toxic effects and inhabiting the growth of others. So, it is always essential to conduct the processes of identification as well as characterization of these microbial species.

Materials and Methods

Study site and location: Chhattisgarh is an agriculture dominant state. Tilda is a Town in Tilda Tehsil in Raipur District of Chhattisgarh State, India. It is located 45 Km towards North from District head-quarters Raipur. It is a Tehsil head quarter. Kohka (3 Km), Biladi (3 Km), Sirve (4 Km), Kota (5 Km), Bahesar (5 Km) are the nearby Villages to Tilda.

The climate of the town is generally characterized by tropical climate, the average annual temperature is 26.8 °C. Precipitation here averages to about 1188 mm. The nature of the soil is generally black, yellow soil. Higher yield efficiency is also found in Tilda. Paddy, maize, jowar, groundnut, gram, and wheat are major crops grown in this area.

The soil samples were collected from selected fields for a period of one year from January 2017 to December 2017.The soil samples were collected at a depth within 10 cm using a metal spatula and 5 to 7 samples were collected randomly and were pooled together. The samples were kept in new polythene bags, sealed and transported to the laboratory for the mycological examination. For the analysis of soil nutrients, one kg of soil was separately collected in polythene bags.

Isolation of soil mycoflora

Dilution plating technique described by Warcup (1950) was used to isolate the mycoflora from soils. Soil sample weighed 1g was diluted in 10 ml of distilled water. One ml of the diluted sample (10 -2 and 10 -3) was poured and spread on the petri plates containing sterilized PDA medium (Potato - 200 grams, dextrose -20 grams, agar -15 grams, distilled water -1000 ml, pH -6.5) in replicates. One percent Streptomycin sulphate solution was added to the medium before pouring into Petri plates for preventing bacterial growth. The inoculated plates were incubated in a dust free cupboard at the room temperature (24±2°C) for 3 - 5 days.

Observation

The colonies growing on PDA plates with different morphology were counted separately. The fungal cultures were then transferred, sub-cultured and the pure cultures were maintained on PDA medium. Fungal morphology was studied microscopically by staining with Lacto-phenol cotton blue and observed under a compound microscope.

Identification

Colony colour and morphology were observed besides hyphal structure, spore size, shapes and spore bearing structures. They were compared with the standard works of Manual of Soil fungi (Gillman, 1957)); Manual of Aspergillus (Raper and Fennell, 1965) and Soil fungi (Domsch et al., 1980).

Analysis of physico-chemical characteristics of the soil

pH, Electrical conductivity, cation exchange capacity, organic carbon, organic matter, available nitrogen, phosphorus, potassium, zinc, copper, iron, manganese, calcium, magnesium, sodium and potassium were analyzed by APHA method (1989).

Results and Discussion

In the present study, a dilution plating method was used for isolating fungi from selected agriculture field soils. Evidently, several authors used dilution plating method for isolation of fungi (Sharma, 2010; Ishaq and Khan, 2011; Pandey et al., 2014; Chandrashekar et al., 2014).

Thirty-eight fungal species representing 15 genera were isolated and identified from selected agriculture field at Tilda (Table 1). Besides the above, maximum number of fungal species belonging to the class Deuteromycetes, followed by Ascomycetes and Phycomycetes were recorded. Likewise, Pandey et al. (2014) accounted 13 genera and 42 fungal species from traditional sugarcane fields in central Uttar Pradesh.

In general, among the 15 genera recorded, the genus *Aspergillus was* (12 species) dominant followed by *Penicillium, Fusarium*, (4 species each), *Curvularia* (3 species), *Trichoderma, Mucor, Cladosporium*

and Rhizopus (2 species). All other genera were represented by one species each (Table 1).

In the present investigation, the genus Aspergillus was dominant followed by Penicillium, and Trichoderma. The data were coincident with the earlier published reports that the constant presence of Aspergillus, Penicillium and Trichoderma from different crop field (Ishaq and Khan, 2011; Ibrahim and Shehu, 2014; Pandey et al., 2014). The physico-chemical characteristics of soil samples showed variation collected from different months. It was also observed that fungal diversity and distribution were correlated with physico-chemical properties of soil. The results showed no significant relationship with pH, electrical conductivity, organic carbon, organic matter, available potassium, available copper, available iron, available manganese, magnesium, available nitrogen, available phosphorus and available zinc. However, Cation exchange capacity, Potassium and Calcium showed positive correlation. The present study clearly revealed that the physico-chemical parameters influenced the diversity and distribution of fungi in agricultural field.

Our results are accordance with the previous findings of Gaddeyya et al. and Rakesh Sharma and Raju (2013). Hence it could be concluded that the diversity and distribution of soil fungi are often influenced by the available nutrients and other physico-chemical conditions of the agricultural fields.

Conclusion

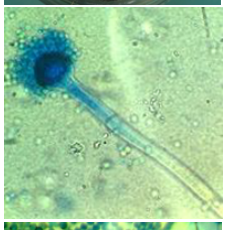
In the present study soil samples of some agriculture fields were studied for screening and detection of fungal diversity. The results obtained clearly indicates that *Aspergillus fumigates*, *Aspergillus niger, Aspergillus flavus* and *Penicillium chrysogenum*, *Trichoderma* were of high occurrence in all selected fields and some other fungi like *Fusarium, Curvularia,* and *Rhizopus* were negligible. Due to high sporulation capacity, *Aspergillus* and *Penicillium* were found to be dominant among the isolates in all of the agricultural sites. *Penicillium* sp produced fungal as well as bacterial antibiotics whereas the *Aspergillus* sp produced various kinds of toxins like aflatoxins, achrotoxins, etc. The growth of other fungal species may be prevented by the former discussed toxins. In the agricultural fields, the mycoflora frequency was regulated by different factors such as humidity, temperature, soil type-texture, vegetation index and organic and inorganic materials.

Table 1: Isolated fungi

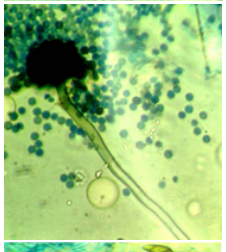
S. No.	Isolated Fungi
1	***Aspergillus* sp.**
	Aspergillus niger *Aspergillus nidulans* *Aspergillus clavatus*
	Aspergillus flavus
	Aspergillus oryzea
	Aspergillus luchuensis
	Aspergillus terreus
	Aspergillus varcicolor
	Aspergillus awamori
	Aspergillus sydowii *Aspergillus fumigatus* *Aspergillus tamarii*
2	***Penicillum* sp.**
	Penicillum digitatum
	Penicillum frequentans
	Penicillum chrysogenum
	Penicillum sp.
3	***Fusarium* sp.**
	Fusarium sp. (Sterile)
	Fusarium oxysporium
	Fusarium solani *Fusarium moniliformae*
4	*Trichoderma* *Trichoderma viride* *Trichoderma* sp.
5	***Mucor* sp.**
	Mucor fragilis
	Mucor sp.
6	*Cladosporium* *Cladosporium herbarum* *Cladosporium* sp.
7	Rhizopus Rhizopus stolonifera Rhizopus orzae
8	Alternaria alternata
9	Curvularia Curvularia clavate Curvularia lunata Curvularia sp.
10	Drechslera sp.
11	*Torula* sp.
12	Humicola sp.
13	*Acremonium* sp.
14	*Gigaspora* sp.
15	*Absidia cerymbifera*

Plate 1: Isolated Fungi

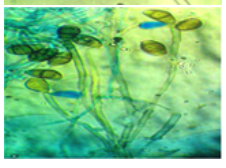
Fungal plate



A. fumigatus



A. niger



Curvularia lunata

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