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PREVALENCE OF SOIL MYCOFLORA IN AGRICULTURAL FIELDS AND ITS ADJOINING DRINKING WATER TUBE WELL SURROUNDINGS IN BETUL DISTRICT, MADHYA PRADESH

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Abstract
We studied the prevalence of fungus in the soils of agricultural (cereals, chilli, coriander, sugarcane, and wheat cultivation) fields and its adjoining drinking water tube well surroundings in Betul District, Madhya Pradesh. A total of 13 soil samples randomly collected from Betul and its subsidiary Multai area were tested. Soil samples were serially diluted and plated on Sabouraud Dextrose Agar incorporated with gentamycin, incubated at 37°C for 4 days. Fungal isolates were stained with lactophenol cotton blue and microscopically identified using standard mycological literature. Among the 6 genus observed, Rhizopus and Aspergillus were more frequent (20% and 10%, respectively) in cereals, sugarcane and wheat cultivation fields. Mucor and Fusarium were found to be higher (10% and 6%, respectively) in coriander and green chilli fields. Candida and Penicillium (each 2%) was found only in the surroundings of drinking water tube wells. We recorded both agriculturally and medically important pathogenic fungus from the tested soils. Indeed, the observed saprophytic fungus may influence the crop productivity and quality of drinking water in these regions. Suitable bio-control and safety measures are needed to eradicate the pathogens to increase the crop productivity as well as the quality of drinking water.

Keywords: Betul district soil mycoflora, prevalence, agricultural fields, drinking water tube wells

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1. INTRODUCTION

Directly or indirectly, humans are in contact with soil during their lifetime. Soil is considered as an excellent growth media for several beneficial and pathogenic microorganisms (Bacteria, fungi, algae, protozoa and viruses) (Griffin, 1972). Microbes involving in symbiotic relationship increases the soil fertility and agricultural crop productivity, maintains ecosystem sustainability, antibiotic and enzyme production, bioremediation, and biodegradation are the distinct beneficial effects. In contrast, pathogens are infectious to humans and domestic animals. Owing to these reasons, there is currently a strong interest to identify and characterize the microorganisms in soil from agricultural fields, water resources and organic farming areas (Van der Heijden *et al*., 2008). Among these, fungi is receiving primary importance due to its readily occurring nature, involvement in biological and biochemical processes occur in soil include turn-over of organic matter, symbiotic and non-symbiotic atmospheric nitrogen fixation, denitrification, and aggregation (Chenu and Stotzky, 2002). Fungal *species* occurs in a region depends on a wide range of external factors includes climate (including nutrition, hygiene and socio-economic circumstances etc. of the inhabitants), susceptibilities and edaphic factors (Sharma and Sharma, 2011). Due to the extreme propagating characteristics of fungal spores, soil-borne fungi are infectious to human, domestic animals, and vegetative crops. Spores of soil-borne fungi transmitted through air interact with any consumable products and drinking water to affect its quality (Shelton *et al*., 2002). An earlier study conducted by Weissman *et al*. (1976), demonstrated the epidemic of gastroenteritis from a contaminated public water supply. Spores also influence the crop productivity,

cause commercial loss to farmers as well as to the economy and development.

Betul district is an agricultural and food treasury of Madhya Pradesh state located in central India. It is situated from 185 km north towards the state capital Bhopal. It covers 10043 km^2, subsidiaries categorized as 8 tehsils viz. Bhainsdehi, Athner, Chicholi, Betul, Shahpur, Multai, Ghodadongari and Amla. All these tehsils were surrounded by forests and highly rich in biodiversity. Half of this district population depends on farming and agriculture. To date, no attempt has been made so far to screen the soil mycoflora in this area. Besides, fungal infections in the agricultural crops are not systematically tested in general. Keeping view of the aforesaid, the present study was aimed to investigate the mycoflora in the soils of agricultural fields and its adjoining drinking water tube well surroundings in Betul District, Madhya Pradesh.

2. MATERIAL AND METHODS

2.1 Study area

For screening soil mycoflora, we selected central Betul and its subsidiary Multai as the study area, due to its soil fertility and renowned for vegetation and plantations, especially vegetables, sugarcane, pulses, and wheat are regular crops cultivated throughout the year.

2.2 Sample collection

A total of 13 soil samples were randomly collected from various agricultural fields and surroundings of drinking water tube wells (TW) in Betul and Multai area, during the summers of 2014 (March – May). The fields were planted to cereals, chilly, coriander, sugarcane, and wheat at the time of sampling. About 250gm of soil (up to12 cm depth) was collected from all the fields, stored in sterile polythene bag then immediately transferred to the laboratory and further processed on the same day.

2.3 Media Preparation and Fungal isolation

Sabouraud Dextrose Agar (SDA) (Hi-Media Laboratories, India) was used for the isolation of fungi. 500 mL of SDA was made according to manufacturer's instruction and sterilized at 121ºC for 30 min. Media was allowed to cool and at room temperature before pouring, 5mL of antibiotic gentamycin solution (Abbott laboratories, India) was added. As prescribed by Waksman (1922), soil dilution method was used for the isolation of fungal strains. Briefly, 1 ml of 10^{-3} soil dilution was plated on SDA, incubated at 37°C for 4 days.

2.4 Fungal staining and identification

To identify the isolated fungal strains, lactophenol cotton blue (LPCB) staining method was used to study the morphology and examined under compound microscope as previously described by us (Senthilkumar *et al*., 2009) (Magnus, Olympus India), using standard mycological literature (Aneja, 2003; Webster and Weber, 2007). Photographs were taken at high power objectives by Sony Cyber shot camera (Sony, Japan). Prevalence of each fungal species was calculated using the below formula

$$\text{Prevalence } (\%) = \frac{\text{Average number of colonies of a species}}{\text{Average number of colonies of overall species}} \times 100$$

Prevalence percentage was calculated and expressed as graphical representation using SPSS 20.0 (SPSS, Chicago, IL, USA).

3. RESULTS AND DISCUSSION

We studied the prevalence of soil mycoflora in agricultural fields and its adjoining drinking water TW in Betul district. Out of 13 soil samples tested, we identified 6 genus viz. *Aspergillus sp*., *Candida sp*., *Fusarium sp*., *Mucor sp.*, *Penicillium sp*., and *Rhizopus sp*. were observed (Table 1).

3 *sp.* of *Aspergillus* (*A. niger*, *A. flavus*, and *A. fumigatus*), 1 *sp.* of *Candida* (*C. parapsilosis*), 1 *sp.* of *Fusarium* (*F. oxysporum*), 2 *sp.* of *Mucor* (*A. glauca* and 1 *sp* unidentified), *2 sp.* of *Penicillium* (*P. chrysogenum* and 1 *sp* unidentified) and *2 sp.* of *Rhizopus* (*R. stolonifer* and 1 *sp* unidentified) through colony and microscopic morphology (Figure 1 – 2).

Table 1. Mycoflora in the soils of agricultural fields and its adjoining drinking water tube well surroundings of Betul and Multai

Sampling	*n*	Planted crop	Genera	*species*	TW	Genera	*species*
Multai							
	2	Cereals	*Rhizopus* *Aspergillus*	UI *A. flavus*	+	*Candida*	*C.parapsilosis*
	1	Chilli	*Mucor* *Fusarium*	*A. glauca* *F. oxysporum*	+	-	-
	1	Coriander	*Mucor* *Fusarium*	UI *F. oxysporum*	+	*Penicillium*	*P. chrysogenum*
	8	Wheat	*Rhizopus* *Aspergillus*	*R. stolonifer* *A. flavus,* *A.fumigatus*	+	*Candida*	*C. parapsilosis*
Betul	1	Sugarcane	*Rhizopus* *Aspergillus*	*R. stolonifer* *A. niger*	-	*Penicillium*	UI

Number of samples collected (*n*); Tube wells (TW); Present (+); Absent (-); Unidentified (UI)

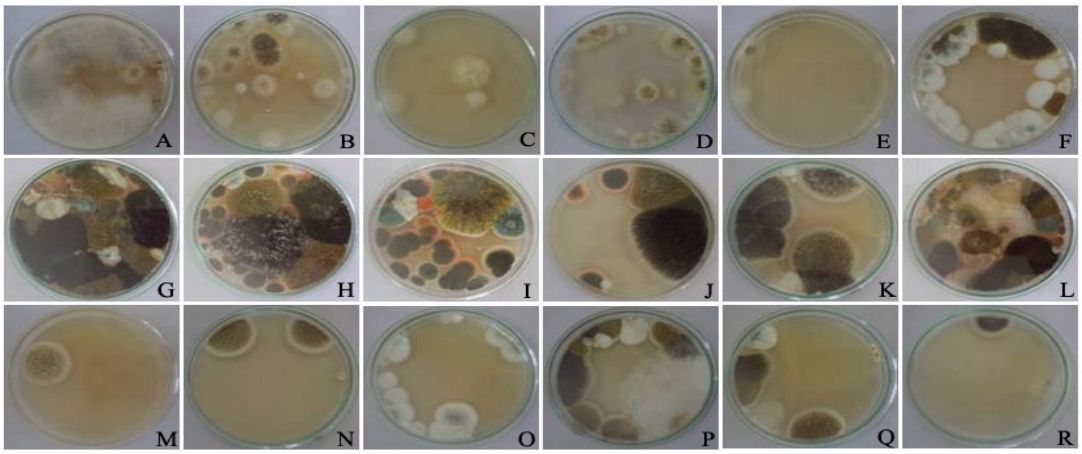

Fig. 1. Fungal isolates from soil samples from Betul (A – F) and Multai (G – R). A – E: surroundings of TW; F – sugarcane field; G – L: Wheat field; J – Cereals; P – Green chilly; Q – R: Coriander

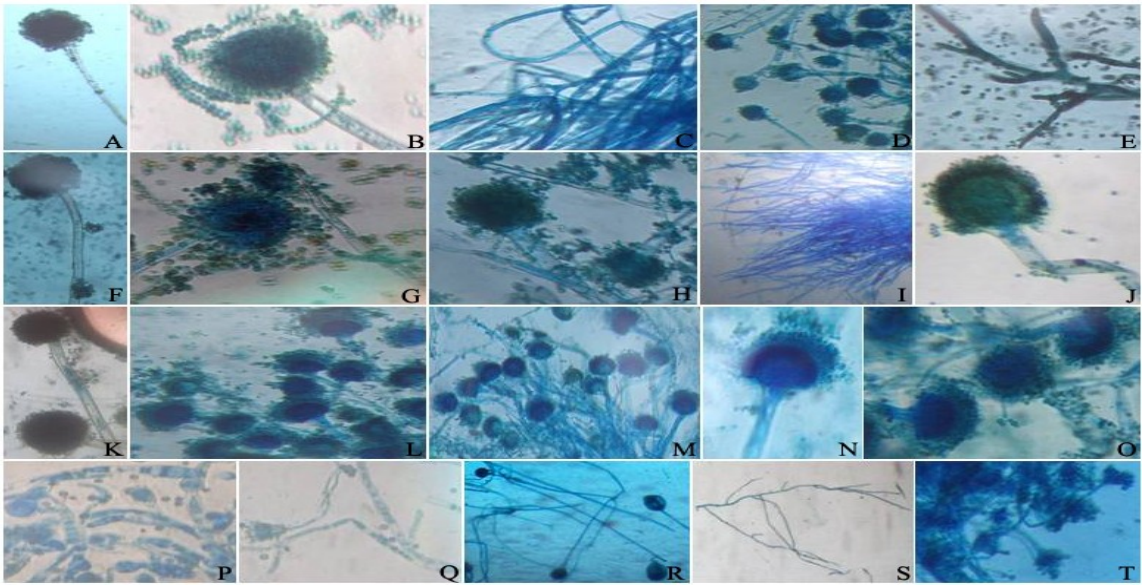

Fig. 2. A, H – *Rhizopus sp.* B, D - *Rhizopus stolonifer, C*, I, Q - *Unidentified*, E – *Serpula lacrymans* (Basidiomycota); F, K – Mucor; G – *Aspergillus niger*; J – *Aspergillus flavus*; L – O - *Aspergillus fumigatus*; P – *Candida parapsilosis* (Ascomycota); R – *Absidia glauca* (Mucorales); S – *Fusarium oxysporum*; T – *Penicillium chrysogenum.*

These observations are supported by an earlier study reported *A. niger*, *A. flavus*, *A. fumigatus, P. chrysogenum* and *R. stolonifer* from paddy, maize, corn, ragi, red gram, cotton, vegetables and sugarcane cultivation fields (Gaddeyya *et al*., 2012; Niharika *et al*., 2013; Rakesh Sharma and Raju, 2013). In specific, Niharika *et al*. (2013) has reported the occurrence of same fungal *species* from sunflower, sesame, capsicum, rice, green gram, sugarcane, ground nut and black gram cultivation fields.

All the 3 *Aspergillus sp. (A. niger*, *A. flavus*, and *A. fumigatus), F. oxysporum, Mucor sp*., *Penicillium sp*., and *R. stolonifer* are renowned for alkaline protease production from soils (Choudhary and Jain, 2012; Kalaskar *et al*., 2014). *A. niger* and *Penicillium sp* is an efficient phosphate solubilizing fungi (Reena *et al*., 2013). Besides, *A. niger* and *A. flavus* significantly involved in heavy metal reduction in industrial effluents (Shivakumar *et al*., 2011). *A. niger*, *A. flavus*, *A. fumigatus, Penicillium sp*., and *R. stolonifer* are antibiotic producing fungi in soils (Makut and Owolewa, 2011). Especially, *P. chrysogenum* is commonly found in agricultural soils (Banik *et al*., 2014).

Among the fungal isolates, *A. fumigatus* and *Candida sp* has significant clinical importance. Spores of *A. fumigatus* can infect human lungs on inhalation (Shelton *et al*., 2002; Panneerselvam and Arumugam, 2012). In humans, *Candida sp*. dermatologically infects the keratin and detaches the cuticle from the nail plate (Baran *et al*., 2003).

The prevalence recorded for *Aspergillus sp*. (10%), *Candida sp*. (02%), *Fusarium sp*. (06%), *Mucor sp*. (10%), *Penicillium sp*. (02%), and *Rhizopus sp*. (20%) (Figure 3).

4. CONCLUSION

Although these observations are preliminary, for the first time we projected the mycoflora in the soils of agricultural fields and its adjoining drinking water tube well surroundings in Betul District. On the other hand, we made an attempt to identify the overall isolated species, the overlapping colonies in the SDA plates made unfeasible to identify few species. These soil-borne mycoflora may influence the crop productivity and quality of drinking water. Suitable bio-control measures are needed to eradicate these pathogens to increase the crop productivity as well as the quality of drinking water in these regions. Further, well-designed studies are needed to authenticate the reported mycoflora in the study area. Now, we are focusing to study the overall agricultural fields in this area for the prevalence of mycoflora.

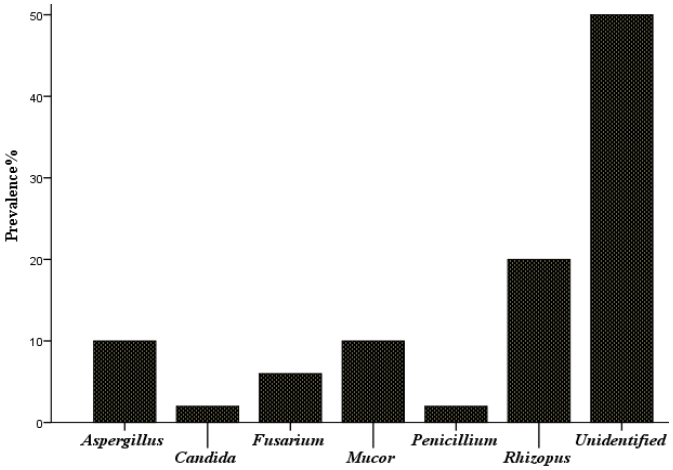

Fig. 3 Distribution of mycoflora in agricultural soils and its adjoining drinking water tube well surroundings

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