

Isolation and identification of Soil fungi from Kota tehsil of Bilaspur District, Chhattisgarh, India.

Pooja Gond^{1*}, Dr. Shweta sao²

¹Research scholar, Department Of Biotechnology, Dr. C.V.Raman University, Kota Bilaspur Chhattisgarh (India)

²Professor, (HOD), Department of Biotechnology, Dr. C.V. raman University, Kota, Bilaspur, Chhattisgarh (India)

Email- pooja4802@gmail.com,

Abstract: - Mycoflora was isolated using soil dilution and soil plate method on Potato Dextrose Agar medium with the addition of the appropriate antibiotic, namely streptomycin. To help identify and describe the mycoflora, fungal guides were consulted. In the soil sample, *Fusarium*, *Rhizopus*, and *Aspergillus* were the most common fungus. They were successful in determining that *Aspergillus niger* was the species that was most common. *Aspergillus niger* was recognized based on its physical and biochemical characteristics, including conformation as shown by lactophenol cotton blue staining and microscopy.

1. Introduction

Fungi are a category of organisms that are omnipresent since they may be found all throughout our surroundings. All types of soil include a diverse microbial flora, which includes fungus. These perform a dynamic role rather than being static. The survival of other creatures on earth depends heavily on the fungal flora, which is one of the key elements of biodiversity. These are also necessary for ecological processes on a global scale (Hawksworth, 2002). Fungi are important members of the soil ecosystem because they break down the organic matter that remains in the soil. (Carlile,2001).

Without a doubt, crop plants take up the soluble mineral nutrients that are present in the soil's inorganic component. However, these minerals are solubilized by decomposers, which are typically fungus or bacteria, either from complex inorganic rock particles or from organic leftovers. As is well known, fungi play a significant role in the ecology of the soil, particularly in agricultural soil, and they are essential to various processes including the breakdown of organic matter and the release of elements through mineralization (Christensen, 1989; Rangaswami, 1998). The biological activity in soil, which is significantly influenced by soil mycoflora, further regulates the recycling of nutrients (Arunchalam, 1997). The amount and kind of organic and inorganic components in the soil have an impact on the number of fungus as well.

Larger quantities of chemical fertilizers and a variety of herbicides and fungicides are employed in contemporary agriculture. They serve a crucial part in the mycoflora, which is very helpful for preserving the soil's fertility and ecological balance (Carroll, 1992; Marschner, 2003; Ayansina, 2006). Agrochemical buildup in soil and water bodies to a hazardous level is bad for both humans and other living things in addition to microbes. The type of nutrients, aeration, moisture, pH, and temperature present in the soil affect this form of fungal density.

The goal of the current study was to investigate the composition of the fungal flora in the paddy fields of Kota, Bilaspur, Chhattisgarh, India. In terms of the colony forming units that were shown on laboratory culture plates, the periodic/monthly Changes in the presence of fungus in soil has been expressed.

2. Materials and Methods

- **Collection Site Study-** In the Chhattisgarh state, the town of Kota lies 34 kilometers (km) north of the district of Bilaspur. It has an average elevation of 1082 feet (330 meters). The towns nearby include Changori, Amali, Khurdur, Pipartarai, and Amane.

- **Method of collection of soil sample-** Three separate agricultural areas each had a different soil sample taken. The soil sample was quickly excavated up to a depth of 12–15 cm using hand trowels and augers. The samples were taken from five points on each site and placed in sterile polythene bags before being transported to the lab and kept at 4°C until the evaluation. The soil samples from the three locations were mixed to form a representative sample (Garrett, 1963).
- **Physiochemical properties of soil-** The acquired soil's physico-chemical properties were listed (Table: 1). The physico-chemical parameters were measured using recognized methods. The pH and moisture content of the soil, as well as its physical and chemical properties, were studied. The soil's texture was evaluated using the wet sieving method (Barbour et al., 1980). The pH of the soil was measured using both Brady (1990)'s electrometric method and a pH meter with a glass electrode. The moisture content of the soil sample was calculated by oven drying the soil and determining the weight loss (Garrett, 1963).

S.No.	Crop field	Soil Colour	pH	Moisture content
1	Paddy crop field	Yellow brown	6.2	60%
2	Paddy crop field	Yellow	6.3	60%
3	Paddy crop field	Yellow	6.2	55%

Table- 1 Physico- chemical properties of soil samples collected from different agriculture fields

- **Isolation of fungi from soil-** The standard methodology described by Warcup (1951) was used throughout the current experiment to isolate fungi. The soil sample, 1.00gm, was dissolved in 10ml of distilled water that had been sterilized. In order to obtain a consistent soil suspension, the soil was continually mixed with the water in an Erlenmeyer glass flask using wrist shaking motions for 15 minutes. The suspension was diluted from 10-1 to 10-6 times, in increments of 10. In petriplates containing PDA culture media supplemented with 0.1% streptomycin, 1.00 ml of 10-3 and 10-4 dilutions were plated. A medium containing the antibiotic was combined to prevent bacterial growth. {**Culture media:** Potato Dextrose Agar (PDA) culture media was utilized for the isolation of soil fungi during present investigation. It has following composition-Dextrose - 20gm, Potato - 50gm, Agar - 20gm, Distilled water - 1000ml, pH - 5.6-5.8}

The isolates were then identified based on their outward colonial look and the morphological/reproductive features visible on their temporary slides. The plates were incubated at 28°C + 2°C for 4-5 days. For every dilution, three duplicates were kept. Following their development on agar plates, fungal species were purified using the single colony isolation technique. For subsequent research, pure cultures were kept on stock and preserved on slants with PDA.

- **Identification of fungi-** The fungus was recognized based on their colony and physical traits. As a mounting fluid, lactophenol cotton blue stain was employed. Fungi were recognized by microscope examination of the slides. The evaluation of the morphological traits took into account the colony's development (length and breadth), the existence or absence of aerial mycelium, the colour of the colony, the presence of wrinkles and furrows, the generation of pigment, etc. The traits were compared to the descriptions found in "A Manual of Soil Fungi" (1957), "Industrial Mycology" (1981), and "Compendium of Soil Fungi" (1980), all written by Gilman.

3. Result

The goal of the study was to identify and grow a soil fungus from the kota area (Bilaspur, Chhattisgarh) in vitro from November to March. A key was utilized to identify isolated fungus using standard literature. For my investigation, 15 isolates were obtained from soil samples. With the aid of a conventional key and a microbiologist, 11 of the 15 isolates were identified. The majority of the isolates of fungi belonged to the genera *Aspergillus* and *Mucor*.



Fig.-1: Plates showing fungal colonies after 4 days of incubation at $28^{\circ}\text{C} \pm 2^{\circ}\text{C}$

S. No.	Size	Colour	Nature of Hyphae	Conidia shape	Name of species
1.	Medium	Black	Non-septate	Rough and irregular	<i>Aspergillus niger</i>
2.	Medium	White	Broad non septate	Ellipsoidal	<i>Mucor sp</i>
3.	Large	Green	Non-septate	Globose	<i>Aspergillus clavatus</i>
4.	Medium	Blue green	Non-septate	Oval	<i>Penicillium crysogenum</i>
5.	Medium	Black	Non-septate	Rough and irregular	<i>Aspergillus niger</i>
6.	Medium	Black	Non-Septate	Oval conidia	<i>Rhizopus stolonifer</i>
7.	Medium	White	Broad non septate	Ellipsoidal	<i>Mucor sp</i>
8.	Small	Grey green	Non-septate	Irregular	<i>Aspergillus fumigates</i>
9.	Medium	Green	Non septate	Globose	<i>Aspergillus flavus</i>
10.	Medium	White	Broad non septate	Ellipsoidal	<i>Mucor sp</i>
11.	Medium	Green	Septate	Oval	<i>Penicillium oxalicum</i>

Numerous soil properties, including pH, organic matter content, and moisture, have an impact on any soil's microbial diversity. The pH range of the soil conditions, which ranged from 6 to 6.5, and the soil textures, according to physicochemical analysis of the soil, determined the fungus population and its diversity in the agricultural fields of the Kota region.

The environment, moisture, organic carbon, and nitrogen all play important roles in determining the proliferation of mycoflora. Tolerance and colonization are significantly impacted at higher moisture levels because soil moisture directly influences the population of fungi in a favorable way. (1999) Adams and co. Studies on current, efficient techniques for removing fungi from their natural settings abound (Labeda, 1992). (Seifert, 1992) presents fundamental methods for separating certain taxonomic groups and habitats, while (Bacon, 1992), which examines endophytic fungi of grasses, provides methods that may be used on different substrates. Bills and (Polishook 1994) have demonstrated the value of particle filtration for the isolation of a range of fungi, in line with what was indicated before.

References

1. Adams, C.P., Bamford, K.M. & Early, M.P.1990. Principles Horticulture (3rd Ed), Utterworth Heineman, p. 25.
2. Bacon, G.W. 1990. Isolation, culture and maintenance of endophytic fungi of grasses In: Isolation of biotechnological organisms from nature. Ed. By P. Labeda, McGraw-Hill, New York, USA.
3. Labeda, D.P. 1996. DNA relatedness among vertical-forming streptomycetes species (formerly streptovorticillium species). *Int. J. Syst. Bacteriol.*, 46:699-703.
4. Polishook, J.D. and Bills, G.F. 1994. Abundance and diversity of microfungi in leaf litter of a lowlandrain forest in Costa Rica. *Mycologia*, 86:187-198.
5. Seifert, K.A 1992. Isolation of filamentous fungi, in: D.P.Labeda biotechnological organisms from nature. MC-Grow- Hill, New York. PP. 21- 51.
6. Hawksworth D. L. 2002. Tropical Mycology Vol.2, Micromycetes- CABI. Pp. 1-11.
7. Carlile, M. J., Watkinson, S. C., Graham, W. G. 2001. The Fungi. Second edition.San Diego, California: Academic Press,.
8. Christensen, M., 1989. A view of fungal ecology. *Mycologia.*, 81:1-19.
9. Rangaswami, G and Bagyaraj,D. J.,1998. Agricultural Microbiology, IInd edition published by Prentice Hall of India Pvt. Ltd. N. Delhi.
10. Arunachalam K. M., Arunachalam, R. S., Tripathi and Pandey, H. N.,1997. *Trop.Ecol.*, 38:333-341.
11. Carroll G. C. and Wicklow D. T., 1992. The Fungal Community: Its Organization and Role in the Ecosystem, New York, Marcel Dekker, Inc.
12. Marschner P., Kandeler E. and Marschner B., 2003. Structure and function of the soil microbial community in a longterm fertilizer experiment, *Soil Biol. Biochem*, 35, 453-461.
13. Ayansina A. D. V. and Oso B. A.,2006. Effect of commonly used Herbicides on soil microflora at two different concentrations, *Afr. J. Biotechnol*, 5(2),129-13.
14. Warcup J.H.,1951. *Trans Br Mycol Soc.*, 34:376-399
15. Gilman, J.C. 1957. A Manual of Soil Fungi. (Second Indian Reprint), Oxford and IBH Publishing Co. New Delhi. India p.450
16. Domsch, H.K., Anderson, T.H. and Gams, W. 1980. Compendium of Soil Fungi. Volume I. Academic Press, A subsidiary of Harcourt Brace Jovanovich, Publishers, London.
17. Onions, A.H.S., Allsopp, D. and Eggins, H.O.W. 1981. Smith's Introduction to Industrial Mycology (Seventh Edition), Edward Arnold Publishing Co.
18. Gaddeyya G, Shiny Niharika P, Bharathi P and Ratna Kumar PK. 2012. Isolation and Identification of soil mycoflora in different crop fields at salurmandal, *Adv. Appl. Sci. Res*, 3 (4): 2020- 2026.
19. Wahegaonkar, N., Salunkhe, S. M., Palsingankar, P. L., Shinde, S. Y. 2011. Diversity of fungi from soils of Aurangabad, M.S., India. In *Annals of Biological Research*, vol. 2, no. 2, p. 198-205.