Pak Heart J 2023:56(03)
ISSN:0048-2706 E-ISSN:2227-9199

Segregation and Screening of Fungal Strains from Soil Contaminated with Pesticides

Rimple Khicher, Ikbal and Neeraj Sethi
Department of Biotechnology, Om Sterling Global University, Hisar, Haryana
rimplebt202@osgu.ac.in
iqbalshah5330@gmail.com
20neerajsethi@gmail.com
Corresponding Author Mail: iqbalshah5330@gmail.com

ABSTRACT
The present investigation was undertaken to find out the fungal diversity in pesticide fields in western Haryana. Twenty soil samples were collected from different locations at four intervals. Fungal strains were isolated from soil samples collected from different zones on PDA medium. The medium underwent treatment with suitable antibiotics, including penicillin and streptomycin, use the soil dilution method and soil plate method. A total of 54 fungal strains from contaminated and 21 fungal colonies from uncontaminated soil were characterized using a variety of identification techniques. Soil samples were also characterized for physiochemical properties. The filamentous fungus from agricultural soils belongs to Ascomycota, which includes seven genera. Zygomycota and Deuteromycota had one genus each. The isolated fungal strains were successfully identified belonging to the genera Aspergillus, Fusarium, Ulocladium, Rhizopus, Humicola, Exserohilum, Drechslera, Curvularia, and Alternaria. Rhizopus, Aspergillus, and Curvularia were the predominant genera. The percentile contribution of the mycoflora was statistically assessed. These fungal species help in the process of biodegradation of pesticides and would be of great help for the farming community at large.

Key words: Aspergillus, Alternaria, Mycoflora, Pesticide, Rhizopus

1. Introduction

The increasing awareness of environmental concerns has resulted in the enforcement of regulatory measures aimed at correcting past flaws and protecting the environment from future pollution and overuse (Shen and Jiang, 2021). The objective of these measures is to preserve the environment and ensure the protection of human health. Various pollutants of concern encompass substances derived from pesticides, which were banned due to the revelation of their hazardous impact on human health (Singh et al., 2019). Among South Asian countries, India is the primary consumer of pesticides, with cotton crops representing the largest share (44.5%) of overall pesticide usage (Ward and Mishra, 2019). Pesticides attach to soil and sediments because of their hydrophobic characteristics. This characteristic imparts enduring stability in soil, sediments and water. The exponential expansion of aquatic and soil ecosystems leads to the accumulation of contaminants in agricultural waste, macrophytes, phytoplankton, fish, vegetables, milk, and dairy products (Pandey and Kumari 2022). Pesticides and their by products tend to build up in the top layer of soil, affecting the quantity of various soil microorganisms and their biochemical processes, including nitrification, ammonification, organic matter decomposition, and nitrogen fixation. (Tripathi et al., 2020).

According to recent figures, almost 99 percent of the pesticides in our country are imported in significant amounts and a highly concentrated form (Sharma et al., 2019). Local manufacturers adulterate and/or combine them with supplementary chemicals to attain the desired composition suitable for local conditions. Unfortunately, these substances frequently demonstrate persistence (Abhilash and Singh, 2009). Despite their discontinuation, some chemicals endure in soils. Sediments can be incorporated into the food chain by direct ingestion or percolating into the water table. Once introduced into the groundwater, these pollutants can seep into drinking water wells and cause health concerns.

Accumulation of noxious compounds in the organisms of mammals and other species occupying higher positions in the food chain might result in enduring health problems (Anderson et al., 2021). Two main aspects contribute to the enduring existence of these compounds in the environment. At first, the necessary conditions for the chemical to break down naturally are not present. The contaminated area may lack the presence of microorganisms capable of biodegrading these toxic compounds. If the necessary microorganisms are present, the biodegradation of the pollutant may be hindered by a limiting factor, such as a lack of nutrients, which prevents the favourable conditions needed for the process (Amenyogbe et al., 2021). The second possibility is that the molecule may demonstrate recalcitrance or resistance to biodegradation.

However, specific microorganisms shown the capacity to survive in environments contaminated with pesticides. Pesticides can exert an influence on the populations of microorganisms residing in the soil (Prudnikova et al., 2021). They can facilitate the proliferation of particular microbes, while simultaneously exerting detrimental effects and constraining the growth of other microorganisms (Naveed et al., 2023). Accurate identification and characterization of these microbial species are essential for examining their potential as candidates for bioremediation (Haider et al., 2023). The organisms possess metabolic capabilities to utilize chemical contaminants as an energy source, leading to the conversion of the contaminants into harmless or less dangerous compounds in most cases. Therefore, the goal of

this research was to isolate and describe fungal strains from locations contaminated with pesticides.

2. Materials and Methods

2.1 Collection of soil samples

To isolate fungal organisms, 20 soil samples were gathered from various locations of Hisar district (in four intervals) that had a history of being treated with pesticides on multiple occasions. Samples were collected in sterile polyethylene zipper bags and then stored at a temperature of 4℃ before being processed.

2.2 Chemicals and reagents

All of the media components and chemicals that were utilized in the research that was conducted were of an analytical quality and were bought from Hi-media laboratory Pvt. Ltd. in Mumbai and Sigma–Aldrich in the United States.

2.3 Characterization of soil samples using physicochemical methods

Physicochemical characteristics include variables such as organic carbon/nitrogen levels, pH, water content, and temperature. Typically, as depth increases, the density of microbial populations generally decreases. This is because the availability of organic carbon and molecular oxygen, which both tend to decrease with depth, diminishes. The temperature and colour of the soil samples were promptly documented on-site. The moisture content, pH, and proportions of organic carbon and organic nitrogen were assessed using established conventional methods. (Kranz et al., 2020)

2.3.1 The moisture content of soil samples

The moisture content of soil samples collected from fields polluted with pesticides was determined by drying 15 g of the samples in an oven at 60°C for 72 hours. The moisture content was then computed (Rasti et al., 2020). The sample's dry weight was determined until it reached a consistent weight. The moisture content was quantified using the following formula:

$$\text{Moisture \%} = \frac{W_1 - W_2}{100} \times 100$$

W1 = Weight of soil before oven drying; W2 = Weight of soil after oven drying

2.3.2 The pH of the soil sample

The soil sample was dried at a temperature of 60°C for 72 hours. It was then ground into a powder using a pestle and mortar and filtered through a sieve with a mesh size of 2 mm. The sieved soil was dissolved in distilled water at a concentration of 2.5 grams per 100 ml and mixed vigorously for 5 minutes at a speed of 120 revolutions per minute using a vortex mixer. The pH of the resulting solution was measured using a digital pH meter. (Ghazali et al., 2020)

2.3.4 Percent organic Carbon / Nitrogen

We measured the soil's mass, which was 1.0 grams, and then transferred it into a conical flask with a capacity of 500 ml, making sure that the flask was dry. A volume of 10 ml of a 1 normal (1N) solution of potassium dichromate ($K_2Cr_2O_7$) was transferred into the flask using a pipette, and then the flask was gently stirred. A volume of 20 ml of concentrated H_2SO_4 solution, containing 1.25% silver sulfate, was introduced into the mixture. The resulting mixture was then agitated by whirling it 2-3 times. The flask was allowed to stand for 30 minutes, and thereafter 200 cc of distilled water was introduced. 10 ml of phosphoric acid and/or 0.5 grams of NaF was incorporated, together with 1 ml of diphenylamine indicator. Perform a titration on the contents of the flask using a 0.5N FAS solution. The FAS (Ferrous Ammonium Sulphate) solution was added into the flask gradually from the burette until the contents of the flask transitioned from blue-violet to green or dull green. After this solution was added slowly, until the color changed to chocolate red. Concurrently, a test is conducted without any soil. (Ledo et al., 2020) Percent organic Carbon was calculated as follows:

$$\text{Volume of 1 N } K_2Cr_2O_7 \text{ consumed/reduced (D)} = \frac{A\,(B-C)}{B}$$

A=amount of K2Cr2O7 used (ml); B=Blank reading; C=sample reading

- **Organic carbon (OC) in soil (%)** = (D × 0.003 × 1.3 × 100) ÷ weight of soil sample in g
- **Organic matter (OM) in soil (%)** = (Organic carbon (%) × 1.724
- **Organic carbon in soil (g/kg)** = Organic carbon (%) × 10
- **Organic carbon (g/ha) in surface 0-15 cm soil** = OC in soil (g/kg) × 2.24 × 106 g/ha=OC in soil (g/kg) × 2.24 × 103 kg/ha
- The following method was used to figure out the soil's organic nitrogen:
- **Organic nitrogen (%)** = 0.862 × % organic carbon

2.4 The isolation and identification of soil fungi

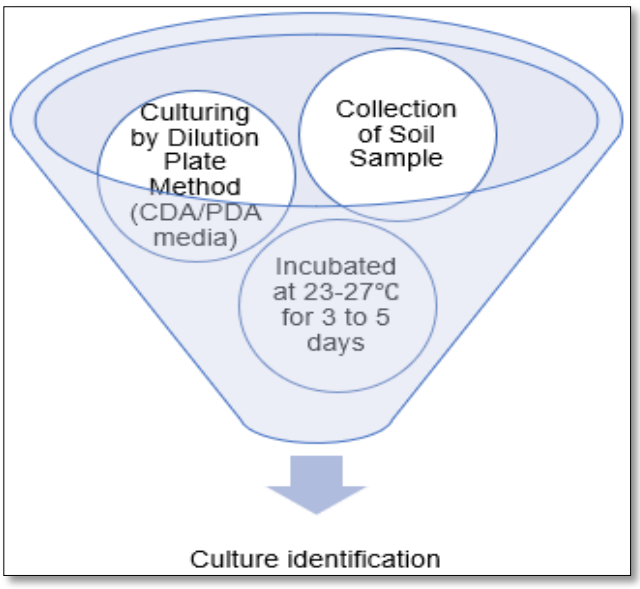

Figure 1: Protocol for Extraction and Screening of fungal strains

2.4.1 Sample preparation and dilution

Aseptically dispensed 10 g of soil into the first dilution bottle (holds 90 mL of diluent), using a top-loading balance and a

sterile spatula. Note the exact wet sample amount. This was the 10^{-1} dilution. A similar sample was used to measure soil dry mass in a tared aluminium pan to express counts (triplicate for precision). The mass was noted after drying the material overnight at 105 °C to a constant mass. The dilution container was mixed vigorously by hand or mechanically for 1 min to 1 hr to break up soil aggregates. The samples underwent a tenfold dilution by transferring a 10.0 mL sample from the center of the dilution container to a new 90 mL bottle. In between bottles were kept on a vortex mixer. (Mohammed et al., 2022) For the enumeration of aerobic heterotrophs in uncontaminated agricultural soils, the sample was diluted to 10^{-9} for the most likely number and 10^{-7} for plate counts using ten-fold serial dilutions.

2.4.2 Potato Dextrose Agar (PDA) plate Technique

The molten PDA medium, which has been cooled to a temperature of 45ºC, was added to the Petri plates. The plate was rotated gently to ensure that the cells were evenly distributed in the medium. The medium was allowed to harden. The inoculation plates were placed in an inverted position and incubated at 37C for 24-48 hours. Observe the plates during this time. (Alam et al., 2023)

Procedure for preparing the lactophenol cotton blue microscopic mount:

A minute quantity of lactophenol cotton blue was administered onto a sterile slide. A small aggregation of fungus, preferably containing spores and structures that bear spores, was inserted into the liquid by delicately positioning it on a sterilised needle that had been heated and subsequently cooled. Careful blending of the stain with the mold structures was done. A cover-glass was positioned gently on top of the preparation, ensuring that no air bubbles were trapped within the sample. The specimen was observed using both the low and high-power objectives of the microscope.

2.4.3 Soil Plate Method or Warcup Method

A total of 0.005 gm of dirt was uniformly distributed around the bottom of the sterile Petri plate. Subsequently, a liquefied agar medium known as PDA was placed onto the soil after it had cooled to a temperature ranging from 40 to 45oC. Subsequently, the dish was delicately rotated to ensure uniform dispersion of the soil particles throughout the medium. The plates were thereafter placed in an incubator set at a temperature of 26oC for 4-5 days. One fungus genus representative was randomly selected from each soil sample for subsequent sub-culturing and research. The subcultures were conserved on Potato Dextrose Agar Slants (Warcup, 1950).

2.4.4 Identification of the Soil Fungi

Typically, the identification of fungal species relies on analyzing the physical traits of the colony and conducting microscopic inspections. The macro morphological characteristics analyzed included the dimensions (length and width) of the colony, the presence or absence of aerial mycelium, the color, the presence of wrinkles and furrows, and any pigment production. Despite advancements in molecular techniques, microscopy, and culture remain widely utilized and indispensable methods for identifying fungal species. The fungi were identified using established protocols and pertinent scientific literature.

2.4.5 Statistical Analysis

The population density is determined in triplicate by quantifying the number of Colony Forming Units (CFU) per gram of soil, considering the variables of dilution. The proportionate contribution of each isolate was calculated by

$$\% \text{ Contribution} = \frac{Total\ No.\ of\ CFU\ of\ an\ indvidual\ Sps}{Total\ No.of\ CFU\ of\ all\ Sps} \text{ x } 100$$

The Results and Discussion

The microorganisms that live in the soil play a crucial role in determining the variety and productivity of the plant community (Dastogeer et al., 2020). The distribution of microflora is significantly influenced by environmental parameters such as the pH of the soil, the amount of moisture present, the temperature, the amount of organic carbon, and the amount of nitrogen (Wang et al., 2021). These are the primary elements that have an impact on the size and variety of the fungus population (Wang et al., 2020).

Research on soil physicochemical parameters is a dynamic and expansive field that encompasses a diverse array of studies aimed at unravelling the intricacies of soil health, fertility, and ecological balance (Paudel, 2023). Investigations into soil fertility and nutrient management delve deep into understanding the relationships between nutrient levels, pH, and organic matter, offering insights into optimizing agricultural practices for sustainable food production. Land degradation studies explore the impact of physicochemical parameters on soil erosion and nutrient loss, guiding strategies for soil remediation and conservation (Ekka et al., 2023). In the context of climate change, researchers examine how altering environmental conditions influence soil moisture, nutrient cycling, and microbial activity, contributing to our understanding of ecosystem resilience. Precision agriculture, integrating technology like sensors and remote sensing, aims to optimize resource use by closely monitoring soil parameters, fostering efficient and sustainable farming practices (Kumar et al., 2022).The intricate web of interactions between soil microorganisms and physicochemical factors is a central focus, providing crucial insights into soil ecology and microbial-driven nutrient cycling (Pan et al., 2023). Urban soil quality research investigates the effects of urbanization on soil health, considering factors like pollution and land use changes (Zhang et al., 2020). Waste management studies explore the impact of organic and inorganic waste on soil parameters, offering sustainable solutions for nutrient enrichment (Verma et al., 2020). Additionally, global initiatives for soil monitoring play a pivotal role in establishing baseline data and formulating strategies for global soil health management. Together, these research endeavors contribute to our comprehensive understanding of soil physicochemical parameters, guiding informed decisions for sustainable land use, agriculture, and environmental conservation on a local and global scale.

Physiochemical properties of soil samples

The current work consisted of investigating the physiochemical parameters of soil that were utilized to separate different kinds of microorganisms. (Table 1)

Colour of the soil

The collected soil samples displayed a variety of hues, which ranged from brown to black

pH

The soil samples also indicated a variety of pH levels, which ranged from 7.89 to 8.65.

Moisture content

The moisture content of the soil varied from 0.45 to 0.89 percent, while the temperature of the soil ranged from 30 to 33℃.

Organic Carbon and Nitrogen Content

There was a range of 0.2568 to 0.4562 for the organic carbon content. The range of organic nitrogen was found to be 0.2876 to 0.3318.

Table 1: Physiochemical Properties of Soil contaminated with pesticides

Sample	Color	Aver Temp℃	Average pH	Average% Moisture	% Organic Carbon	Average % Organic Carbon
S1	Gray	32.3±0.37	8.22±0.01	0.51±0.0436	0.401±0.00026	0.309±0.00035
S2	Brown	30.9±0.26	8.34±0.02	0.43±0.0265	0.412±0.00026	0.287±0.00017
S3	Gray	31.2±0.2	8.45±0.01	0.44±0.0200	0.456±0.00020	0.329±0.00020
S4	Black	33±0.5	8.67±0.03	0.59±0.0265	0.365±0.00010	0.32±0.00046
S5	Brown	32±1.32	8.34±0.01	0.76±0.0361	0.311±0.00020	0.352±0.00026
S6	Brown	30.5±0.3	8.55±0.01	0.78±0.0436	0.299±0.00010	0.302±0.00044
S7	Dark Brown	30.6±0.26	8.12±0.03	0.22±0.0173	0.356±0.00044	0.310±0.00010
S8	Gray	31.7±0.26	8.1±0.02	0.54±0.0100	0.345±0.00026	0.287±0.00046
S9	Black	33.6±0.32	8.23±0.04	0.65±0.0300	0.322±0.00020	0.272±0.00026
S10	Brown	31.4±0.1	8.1±0.03	0.68±0.0300	0.409±0.00010	0.317±0.00026
S11	Brown	30.2±0.17	8.56±0.02	0.56±0.0173	0.387±0.00044	0.312±0.00010
S12	Gray	32.6±0.17	8.22±0.02	0.54±0.0361	0.328±0.00030	0.321±0.00053
S13	Brown	31.1±0.1	8.5±0.01	0.42±0.0200	0.412±0.00017	0.303±0.00026
S14	Black	32.2±0.43	8.26±0.02	0.43±0.0265	0.361±0.00078	0.310±0.00020
S15	Dark Brown	33.1±0.1	8.43±0.05	0.67±0.0265	0.256±0.00056	0.307±0.00026
S16	Brown	30.8±0.2	8.45±0.04	0.7±0.0200	0.261±0.00035	0.302±0.00026
S17	Black	30.5±0.1	8.65±0.05	0.56±0.0361	0.281±0.00044	0.331±0.00010
S18	Dark Brown	32.1±0.1	8.44±0.02	0.55±0.0100	0.256±0.00036	0.297±0.00026
S19	Gray	30.2±0.1	8.56±0.04	0.44±0.0436	0.356±0.00053	0.307±0.00026
S20	Black	30.5±0.36	7.89±0.02	0.34±0.0173	0.356±0.00061	0.311±0.00026

Isolation and Characterisation of Fungal Species

Observations were made of the microflora that was present in the soil of Hisar district. Both the soil dilution plate and the soil plate procedures were utilized to successfully isolate fungus during this inquiry. The total number of fungal colonies that were isolated on Petri plates that contained PDA medium was 54. To isolate fungi during the current experiment, the soil dilution plate and the soil plate method were utilized, as was mentioned earlier. When compared to dilution plates, soil plates were used to isolate a greater number of species and colonies. Furthermore, the total number of species isolated declined when the dilutions of the samples were raised. The culture was purified through either the isolation of a single spore or the cultivation of the hyphal tips. After this, the culture was transferred to fresh agar slants which contained PDA media. On the dilution plates, the majority of the fungal forms that produce a large number of spores were found in abundance.

Fungi are the most important decomposers of dead organic materials and provide a considerable contribution to the recycling of nutrients in both natural and modified ecosystems (Raza et al., 2023). To determine the degree of fungal variety, a total of 20 soil samples were collected from distinct locations. The findings of the study revealed the existence of 15 different species of fungi, all of which were separated and described using PDA plates (Table: 2). It was mentioned by numerous workers who have worked with it earlier that PDA medium is the finest media for mycelia growth due to its straightforward formulation and the fact that it has the potential to support a wide range of fungal growth (Buffi et al., 2023). PDA medium is the form of culture media that is used the most frequently. Through the utilization of authentic manuals of soil fungus, the isolations were characterized up to the genus level and down to the species level (Qureshi, et al., 2023). This was accomplished by utilizing the macro-morphological and micro-morphological characteristics of the isolates.

As can be seen in Table 1, the findings demonstrated that the physicochemical parameters of the soil samples collected from various locations exhibited a distinct degree of variance. In addition to the number of soil microorganisms, the variety of those microbes is also influenced by several soil parameters, such as the amount of organic matter, the pH level, and the amount of moisture present in the soil (Lehmann et al., 2020). Therefore, it is of the utmost importance to investigate the connection between the physicochemical characteristics of the soil and the amounts of native microorganisms that are present in the soil. When it comes to the activity of the microorganisms that are present in the soil, the presence of moisture in the soil serves as a solvent and is necessary. The presence of an adequate quantity of organic matter and moisture in the soil is necessary to maintain a robust microbial population in the soil system. At this point, the investigation is focusing on the most significant aspects of the circumstance. The current analysis was carried out to identify the significant physicochemical parameters of the soils. These parameters were thereafter applied to calculate the natural fungal density.

Isolation and characterization of Fungal strains

Through the assessment of twenty soil samples treated with pesticides, a total of 54 fungal isolates were discovered, consisting of 14 known species and 5 undetermined species. The procurement of each fungal isolate was conducted in uncontaminated cultures, and the methods employed were considered conventional. Photomicrographs were obtained for each of the discovered fungal isolates to assist in their identification. Figures 2 and 3 depict the spore-forming structures of these isolates and Table 2 represents 54 fungal isolates with their percent contribution.

Table 2: Frequency of mycoflora in different Agricultural Fields having Pesticides as on Potato Dextrose Agar Medium

Fungal Genus	Fungal Species	Average Number of Individual Colonies	% Contribution
Aspergillus	*A. nidulans*	6	11.11
	A. sclerotiorum	2	3.70
	A. ochraceus	3	5.55
	A. oryzae	2	3.70
	A. flavus	1	1.85
	A. niger	5	9.25
	A. alliaceus	5	9.25
	A. candidus	2	3.70
	A. fumigatus	1	1.85
	Unknown	3	5.55

Fusarium	*F. acuminatum*	1	1.85
Ulocladium	Unknown	3	5.55
Rhizopus	Unknown	7	12.96
Humicola	*Unknown*	2	3.70
Exserohilum	*E. turcicum*	1	1.85
Drechslera	*D. australiensis*	2	3.70
	D. halodes	1	1.85
Curvularia	*Curvularia asianensis*	4	7.40
Alternaria	Unknown	3	5.55
Total number of Colonies		**54**	

Table 3: Frequency of mycoflora in different Fields without pesticides as on Potato Dextrose Agar Medium

Fungal Genus	**Fungal Species**	**Average Number of Individual Colonies**	**% Contribution**
Aspergillus	*A. sclerotiorum*	1	4.76
	A. oryzae	1	4.76
	A. niger	3	14.28
	A. alliaceus	2	9.5
	A. candidus	2	9.5
	Unknown	7	33.33
Fusarium	*F. acuminatum*	1	4.76
Rhizopus	Unknown	3	14.28
Curvularia	*Unknown*	1	4.76
Total number of Colonies		**21**	

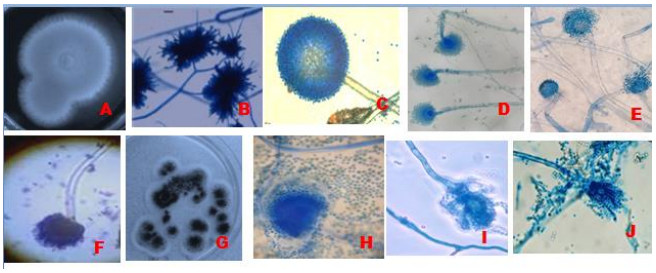

Figure 2: Species belonging to Aspergillus discovered from soil samples. A) *A. Nidulans,* ***B)*** *A. Sclerotiorum,* ***C)*** *A. Ochraceus,* ***D)*** *A. Oryzae,* ***E)*** *A. Flavus,* ***F)*** *A. Niger,* ***G)*** *A. Alliaceus,* ***H)*** *A. Candidus,* ***I)*** *A. Fumigates,* ***J)*** Unknown

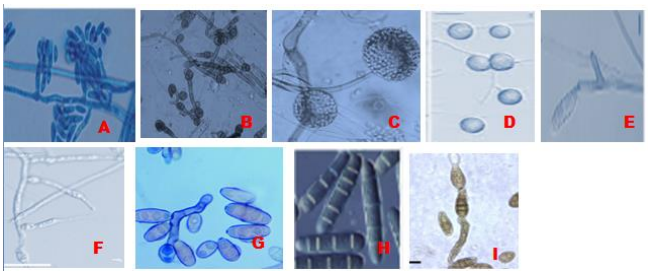

Figure 3: Species belonging to some other genus discovered from soil samples.

A) *Fusarium acuminatum,* ***B)*** *Ulocladium spp.,* ***C)*** *Rhizopus spp.,* ***D)*** *Humicola spp.,* ***E)*** *Exserohilum turcicum,* ***G)*** *Drechslera australiensis* ***F)*** *Drechslera halodes,* ***G)*** *Curvularia asianensis,* ***H)*** *Alternaria spp.*

In case of contaminated soil, the filamentous fungus was found to belong to the division Ascomycota, which comprised seven different genera (Aspergillus, Curvularia, Exserohilum, Fusarium, Ulocladium, Drechslera, and Humicola). Additionally, the division Zygomycota consisted of one genus (Rhizopus), and the division Deuteromycota consisted of one genus (Alternaria). Individuals belonging to genera such as Rhizopus, Aspergillus, and Curvularia were the most frequent. (Table 2)

The filamentous fungus isolated from uncontaminated agricultural soils was found to belong to the division Ascomycota, which comprised three different genera (Aspergillus, Curvularia, Fusarium). Additionally, the division Zygomycota consisted of one genus (Rhizopus). Individuals belonging to genera such as Rhizopus, and Aspergillus were the most frequent. (Table 3)

Raja et al., 2017 conducted a study to ascertain the fungus diversity in soil samples. They obtained 25 isolates from the soil samples. The majority of the fungal isolates consisted of species from the genera Aspergillus and Mucor. The soil fungi that have been identified are Aspergillus niger, Aspergillus clavatus, Aspergillus sydowii, Aspergillus variabilis, Aspergillus fumigatus, Penicillium chrysogenum, Colletotrichum gloeosporioides senu lato, and Mucor sp. The species listed are Rhizopus stolonifer, Rhizopus oryzae, Cunninghamella bertholletiae, Scopulariopsis brumptii, and Cladophialophora sp. Among the species identified on campus, the Keratinophilic fungus Aspergillus niger were found to be the most abundant, followed by Mucor sp.
In another study out of the 800 soil samples analysed, a total of 727 isolates, comprising 16 species belonging to 11 different genera were analysed. The distribution of fungal species in the sample consisted of Curvularia spp., Fusarium spp. and Ulocladium spp. (Kachuei et al., 2012).

In further research, isolated fungal cultures were tested for lipolytic enzyme synthesis using tributyrin agar. The most lipase-producing strain was *Rhizopus oryzae* ZAC3 (NCBI number KX035094) was isolated from soil. On day four, lipase synthesis peaked at pH 5.0 and 45°C. Olive oil, xylose, and yeast extract were the best carbon and nitrogen inducers for lipase development. Optimal conditions enhanced lipase synthesis 2.02-fold. ZAC3 lipase from *Rhizopus oryzae* is used in industry and biotechnology. (Ayinla et al., 2017)

Depending on the availability of nutrients and the appropriate environmental conditions, fungus can dwell in such soil either as latent propagules or as active mycelia. This is made possible by the soil's particular characteristics (Zhang et al. 2021). There is a greater quantity of dormant propagules than there are active mycelia. Some of these species have been reported as common isolates from polluted soil that was caused by pesticides. There is evidence that specific species are utilized for the biodegradation of xenobiotics, and some of these species have been reported previously (Miglani et al., 2022). There is also evidence that these animals are being exploited for their resources. The rapid growth and simultaneous appearance of these species demonstrate that the fungal strains in question have a high degree of adaptation to the soil in which they are present (Nji et al., 2023). There will be extra utilization of the fungal species that simultaneously evolved for the destruction of the pesticides that are routinely applied.

Conclusion

In this study, 54 fungal colonies were found in pesticide-contaminated agricultural soil and 21 fungal colonies were found in non-contaminated soil. Staining with lactophenol cotton blue identified 19 fungal species from 9 genera in the phyla ascomycota, deuteromycota, and zygomycota by morphological and microscopic investigation. Most indigenous fungi can efficiently grow and appear simultaneously, indicating their ability to adapt to xenobiotic substances as growth and energy substrates. Further research will evaluate the ability of these fungi to breakdown xenobiotic chemicals, including routinely used pesticides.

References

Abhilash, P. C., & Singh, N. (2009). Pesticide use and application: an Indian scenario. *Journal of hazardous materials*, *165*(1-3), 1-12.

Alam, R., Mahmood, R. A., Islam, S., Ardiati, F. C., Solihat, N. N., Alam, M. B., ... & Kim, S. (2023). Understanding the biodegradation pathways of azo dyes by immobilized white-rot fungus, Trametes hirsuta D7, using UPLC-PDA-FTICR MS supported by in silico simulations and toxicity assessment. *Chemosphere*, *313*, 137505.

Amenyogbe, E., Huang, J. S., Chen, G., & Wang, Z. (2021). An overview of the pesticides' impacts on fishes and humans. *International Journal of Aquatic Biology*, *9*(1), 55-65.

Anderson, D. M., Fensin, E., Gobler, C. J., Hoeglund, A. E., Hubbard, K. A., Kulis, D. M., ... & Trainer, V. L. (2021). Marine harmful algal blooms (HABs) in the United States: History, current status and future trends. *Harmful Algae*, *102*, 101975.

Ayinla, Z. A., Ademakinwa, A. N., & Agboola, F. K. (2017). Studies on the optimization of lipase production by Rhizopus sp. ZAC3 isolated from the contaminated soil of a palm oil processing shed.

Buffi, M., Cailleau, G., Kuhn, T., Li Richter, X. Y., Stanley, C. E., Wick, L. Y., ... & Junier, P. (2023). Fungal drops: a novel approach for macro-and microscopic analyses of fungal mycelial growth. *Microlife*, *4*, uqad042.

Dastogeer, K. M., Tumpa, F. H., Sultana, A., Akter, M. A., & Chakraborty, A. (2020). Plant microbiome–an account of the factors that shape community composition and diversity. *Current Plant Biology*, *23*, 100161.

Ekka, P., Patra, S., Upreti, M., Kumar, G., Kumar, A., & Saikia, P. (2023). Land Degradation and Its Impacts on Biodiversity and Ecosystem Services. *Land and Environmental Management through Forestry*, 77-101.

Ghazali, M. F., Wikantika, K., Harto, A. B., & Kondoh, A. (2020). Generating soil salinity, soil moisture, soil pH from satellite imagery and its analysis. *Information Processing in Agriculture*, *7*(2), 294-306.

Haider, A., Ringer, M., Kotroczó, Z., Mohácsi-Farkas, C., & Kocsis, T. (2023). The Importance of Protein Fingerprints in Bacterial Identification: The Maldi-Tof Technique. *Journal of Environmental Geography*, *16*(1-4), 38-45.

Kachuei, R., Emami, M., Naeimi, B., & Diba, K. (2012). Isolation of keratinophilic fungi from soil in Isfahan province, Iran. *Journal de Mycologie Medicale*, *22*(1), 8-13.

Kranz, C. N., McLaughlin, R. A., Johnson, A., Miller, G., & Heitman, J. L. (2020). The effects of compost incorporation on soil physical properties in urban soils–A concise review. *Journal of Environmental Management*, *261*, 110209.

Kumar, S., Meena, R. S., Sheoran, S., Jangir, C. K., Jhariya, M. K., Banerjee, A., & Raj, A. (2022). Remote sensing for agriculture and resource management. In *Natural Resources Conservation and Advances for Sustainability* (pp. 91-135). Elsevier.

Ledo, A., Smith, P., Zerihun, A., Whitaker, J., Vicente-Vicente, J. L., Qin, Z., ... & Hillier, J. (2020). Changes in soil organic carbon under perennial crops. *Global change biology*, *26*(7), 4158-4168.

Lehmann, A., Zheng, W., Ryo, M., Soutschek, K., Roy, J., Rongstock, R., ... & Rillig, M. C. (2020). Fungal traits important for soil aggregation. *Frontiers in microbiology*, *10*, 2904.

Miglani, R., Parveen, N., Kumar, A., Ansari, M. A., Khanna, S., Rawat, G., ... & Ansari, M. N. (2022). Degradation of xenobiotic pollutants: an environmentally sustainable approach. *Metabolites*, *12*(9), 818.

Mohammed, F. S., Kına, E., Uysal, İ., Mencik, K., Dogan, M., Pehlivan, M., & Sevindik, M. (2022). Antioxidant and antimicrobial activities of ethanol extract of Lepidium spinosum. *Turkish Journal of Agriculture-Food Science and Technology*, *10*(6), 1116-1119.

Naveed, M., Naveed, R., Aziz, T., Iqbal, F., Hassan, A., Saleem, A., ... & Alasmari, A. F. (2023). Exploring the potential application of peroxidase enzyme from Acinetobacter baumannii as an eco-friendly agent for the bioremediation of the highly noxious pyrethroid compounds through molecular docking analysis. *Biomass Conversion and Biorefinery*, 1-19.

Nji, Q. N., Babalola, O. O., & Mwanza, M. (2023). Soil Aspergillus Species, Pathogenicity and Control Perspectives. *Journal of Fungi*, *9*(7), 766.

Pan, C., Yu, W., Sun, C., Guo, J., Yu, Y., & Li, X. (2023). Saprotrophic fungi buffer the adverse effects of soil acidification on the soil nutrient supply ability of Chinese fir (Cunninghamia lanceolata) plantations. *European Journal of Soil Biology*, *114*, 103462.

Pandey, S., & Kumari, N. (2022). Sustainable utilization and management of agricultural and kitchen waste. *Agricultural and Kitchen Waste*, 127-163.

Paudel, S. (2023). *Understanding How Agricultural Practices Shape Microbial Dynamics and Soil Health* (Doctoral dissertation, North Dakota State University).

Prudnikova, S., Streltsova, N., & Volova, T. (2021). The effect of the pesticide delivery method on the microbial community of field soil. *Environmental Science and Pollution Research*, *28*, 8681-8697.

Qureshi, A. K., Farooq, U., Shakeel, Q., Ali, S., Ashiq, S., Shahzad, S., ... & Manachini, B. (2023). The Green Synthesis of Silver Nanoparticles from Avena fatua Extract: Antifungal Activity against Fusarium oxysporum f. sp. lycopersici. *Pathogens*, *12*(10), 1247.

Raja, M., Praveena, G., & William, S. J. (2017). Isolation and identification of fungi from soil in Loyola college campus, Chennai, India. *Int J Curr Microbiol App Sci*, *6*(2), 1789-95.

Rasti, A., Pineda, M., & Razavi, M. (2020). Assessment of soil moisture content measurement methods: Conventional laboratory oven versus halogen moisture analyzer. *Journal of Soil and Water Science*, *4*(1), 151-160.

Raza, T., Qadir, M. F., Khan, K. S., Eash, N. S., Yousuf, M., Chatterjee, S., ... & Oetting, J. N. (2023). Unrevealing the potential of microbes in decomposition of organic matter and release of carbon in the ecosystem. *Journal of Environmental Management*, *344*, 118529.

Sharma, A., Kumar, V., Shahzad, B., Tanveer, M., Sidhu, G. P. S., Handa, N., ... & Thukral, A. K. (2019). Worldwide pesticide usage and its impacts on ecosystem. *SN Applied Sciences*, *1*, 1-16.

Shen, W., & Jiang, D. (2021). Making authoritarian environmentalism accountable? Understanding China's new reforms on environmental governance. *The journal of environment & development*, *30*(1), 41-67.

Singh, P. K., Singh, R. P., Singh, P., & Singh, R. L. (2019). Food hazards: Physical, chemical, and biological. In *Food safety and human health* (pp. 15-65). Academic Press.

Tripathi, S., Srivastava, P., Devi, R. S., & Bhadouria, R. (2020). Influence of synthetic fertilizers and pesticides on soil

health and soil microbiology. In *Agrochemicals detection, treatment and remediation* (pp. 25-54). Butterworth-Heinemann.

Verma, B. C., Pramanik, P., & Bhaduri, D. (2020). Organic fertilizers for sustainable soil and environmental management. *Nutrient dynamics for sustainable crop production*, 289-313.

Wang, B., An, S., Liang, C., Liu, Y., & Kuzyakov, Y. (2021). Microbial necromass as the source of soil organic carbon in global ecosystems. *Soil Biology and Biochemistry*, *162*, 108422.

Wang, H., Yan, S., Ren, T., Yuan, Y., Kuang, G., Wang, B., ... & Liu, G. (2020). Novel environmental factors affecting microbial responses and physicochemical properties by sequentially applied biochar in black soil. *Environmental Science and Pollution Research*, *27*, 37432-37443.

Warcup, J. H. (1950). The soil-plate method for isolation of fungi from soil. *Nature*, *166*(4211), 117-118.

Ward, A., & Mishra, A. (2019). Addressing sustainability issues with voluntary standards and codes: a closer look at cotton production in India. *Business Responsibility and Sustainability in India: Sectoral Analysis of Voluntary Governance Initiatives*, 161-193.

Zhang, R., Zhao, X., Zhang, C., & Li, J. (2020). Impact of rapid and intensive land use/land cover change on soil properties in arid regions: a case study of Lanzhou new area, China. *Sustainability*, *12*(21), 9226.

Zhang, Z., Zhang, J., & Jiao, S. (2021). Fungi show broader environmental thresholds in wet than dry agricultural soils with distinct biogeographic patterns. *Science of the Total Environment*, *750*, 141761.