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ISOLATION AND IDENTIFICATION OF FUNGAL SPECIES IN PESTICIDE-CONTAMINATED SOIL FROM ABO-OBOSI, ANAMBRA

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ABSTRACT: The present investigation was undertaken to identify the fungal diversity in pesticide agricultural farmland in Abo-Obosi, Anambra State, Nigeria. Fungal species were isolated from soil samples collected from different points within the farmland on a PDA medium using the serial dilution method and spread plate cultural method. The medium underwent treatment with suitable antibiotics, including penicillin and chloramphenicol. A total of 8 fungal species from the contaminated soil were characterized by their morphological and microscopic features. The isolated fungal species were successfully identified belonging to five genera; *Aspergillus* sp., *Rhizopus* sp., *Alternaria* sp., *Geotrichum* sp., *Cladiosporum* sp., and *Fusarium* sp. The study reveal that the fungi isolates have biodegradative abilities on two tested pesticides (Glyphosate marked as pesticide A and Paraquat glusifonate marked as pesticide B). Isolate 6 (*Geotrichum* sp.) had the highest degradation potential with 63.48% and 62.74 % on pesticides A and B respectively. However, isolate 5 (*Aspergillus* sp.) had the lowest degradation potential with 6.33% on pesticide A and 10.77% on pesticide B respectively. All isolates showed resistance potential against both pesticides with pesticide B being most susceptible. This study has shown that there exists a diverse pool of fungi species in the soil ecosystem which can remediate soil polluted by the excessive use of pesticides in modern cum commercial farming systems. Enhancing the ability of these indigenous organisms through various bioremediation technologies will play a key role in keeping the soil ecosystem safe for beneficial microorganisms while ensuring sustainable development.

Keywords: Isolation, Identification, Fungal Species Pesticide, Soil.

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1. INTRODUCTION

Pesticides are synthetic chemical compounds that are used to eliminate, rodents, insects, fungi, and weeds. They include herbicides, insecticides, nematicides, fungicides, molluscicides, rodenticides, plant growth regulators, and other compounds [26]. It is generally used to inhibit disease spread by vectors, including crop protection, and food preservation, and also plays relevant roles in commercial and food-based industrial processes and practices, such as in aquaculture, agriculture, food processing, preservation, and storage [22]. The increase in world population over the years has led to an increasing need and demand for food production. The Food and Agricultural Organization (FAO), of the United Nations, has advocated for the increase in global food production to about 70% to meet the demand of the growing world population. One major challenge of food production is the unavailability of commensurate land space to expand agricultural activities [21]. This challenge has therefore put significant pressure on available agricultural systems such that food production is being achieved with the same resources such as land space, water, etc. Also, the increase in food production has led to the corresponding increase in the application of fertilizers, pesticides, herbicides, insecticides, fungicides, and other soil amendments in much higher quantities than were previously used. Pesticides have now become an important part of our modern agricultural practices used to protect crop plants, stored grain, and flowers as well as to eliminate the pests transmitting infectious diseases. The application of pesticides enhances food productivity by reducing pest infestation and improving supply conditions [19]. However, its indiscriminate use, without following the technical recommendations has been known to have caused serious impacts on the environment and human health [3]. It has been reported that exposures to pesticides are increasingly linked to various health concerns such as immune suppression, hormonal imbalance, reduced intelligence, reproductive abnormalities, and cancerous growth. It has been estimated that in the global space, nearly $ 38 billion is expended on pesticides annually [13]. Researchers and manufacturers are advocating and designing new formulations of pesticides to meet the global need. The applied pesticides should be highly specific in action by being harmful to only target organisms, they should be biodegradable and eco-friendly [20]. Regrettably, this hasn't been the case as most of the available pesticides are non-specific and may kill organisms that are beneficial to the ecosystem. Generally, it has been predicted that only about 0.1% of the pesticides reach the target organisms and the remaining sum contaminates

the surrounding environment [6]. Consistent application of persistent and non-biodegradable pesticides has contaminated various water, air, and soil ecosystem components. Pesticides have also integrated into the ecological food chain and have bioaccumulated in the higher trophic levels. Acute and chronic diseases affecting humans have been recently attributed to pesticide exposure [11].

This current study aims to isolate and identify fungi species that are Indigenous in pesticide-polluted soil in selected farmlands within Abo-Obosi, Anambra state, Nigeria.

2. MATERIALS AND METHODS

2.1. Sample Collection

Soil samples were collected from six (6) points in Abo-Obosi farmland used to cultivate annual crops. Inorganic fertilizers, herbicides, and insecticides have been regularly applied to cropland to improve yield. The sampling method as described by Makut and Mohammed [8] was used. The samples were collected from a soil depth of 5-10 cm using a sterile hand trowel and were transported to the laboratory in a sterile polyethylene bag and stored at 4^{o}C before use. Before the experiment was set up, the soil samples were air-dried and passed through a 2 mm mesh sieve to remove debris and large particles of soil [12].

Pesticides/Herbicide

The pesticides/herbicides used in this study are Glyphosate marked as pesticide **A** and Paraquat glusifonate marked as pesticide **B**.

Isolation of Fungi

By adopting the modified method by Uchendu and Mbonu [25], 10 g of the soil samples were aseptically pipetted and introduced into 90 mL of sterile physiological saline to form an aliquot. A tenfold serial dilution of the slurry suspension was carried out by transferring 1 mL of each aliquot into test tubes containing 9 mL of sterile physiological saline arranged serially in the order $10^{-1} – 10^{-4}$. Zero-point one (0.1) mL of 10^{-4} dilution was spread over culture plates containing sterile Potato Dextrose Agar (PDA) (prepared according to the manufacturer's description), supplemented with 100 mg ml^{-1} chloramphenicol and 15 mg ml^{-1} of penicillin to inhibit bacterial growth. The samples were uniformly spread on the surface of the medium with a sterile glass rod. All the plates were incubated at 28 ± 2 °C for 4 - 7 days. The emerging fungi were transferred to fresh PDA plates, incubated at the conditions above, and periodically checked for purity. The predominant forms of fungal growth were tentatively selected and given a laboratory-isolated number after purification.

2.2. Pre–Screening of the Biodegradative Capabilities of the Isolate Using Dichlorophenolindophenol (DCPIP) Colorimetric Method

The DCPIP indicator colorimetric test was used to determine the herbicide-degrading ability of the isolated predominant fungi from the pesticide-contaminated samples. The colorimetric assay was carried out according to the methods of Bidoia et al. [4] and Prathyusha et al. [15]. The microbial

culture was reactivated after 48 h and inoculated to 50 mL of Bushnell Haas (BH) medium at 35 °C for biomass growth. (The BH medium contents are, $MgSO_4$, 0.2g; $CaCl_2$, 0.02g; KH_2PO_4, 1.0g; K_2HPO_4, 1.0g; NH_4NO_3, 1.0g; FeCl3, 0.05g;). After 48 h in BH medium, individual fungal cells were inoculated into tubes along with DCPIP indicator (0.1 % w/v) and the 1 % herbicide under analysis. The two controls were without organisms and herbicides, respectively. After proper incubation, the color was observed and the absorbance of each tube was taken at 600 nm and recorded. The absorbance values were used to calculate the percentage degradation for each organism as follows:

$$\text{Percentage degradation (\%)} = \frac{\text{Absorbance of control - Absorbance of test}}{\text{Absorbance of control}} \times \frac{100}{1}$$

2.3. Evaluation of the Herbicide Resistance Potentials of the Selected Strain

The isolates were screened for their potential to tolerate herbicide by adopting the modified methods of Rani et al. [17] and Tkaczuk et al. [23]. Initially, the strains of fungi were grown on culture plates pre-filled with Potato Dextrose Agar (PDA) and incubated at 28 ± 2 °C for 7 days. Following incubation, mycelial agar plugs (6 mm^2) were cut approximately 5 mm from the colony margin and centrally inoculated on the surfaces of prepared sterile potato dextrose agar (PDA) plates containing increasing herbicide (A and B) concentrations of 10 ppm, 50 ppm, 100 ppm, 200 ppm, and 500 ppm. The plates were incubated as previously described above and the colony diameter was measured at 1, 2, and 3 days after inoculation using a calibrated meter rule. The herbicide-containing experiment and the control experiment were replicated twice [9].

2.4. Characterization and Identification of the Most Tolerant Fungal Strain

The selected dominant and herbicide-resistant fungal strains were preliminary identified according to their macroscopic and microscopic characteristics as follows:

Cultural and microscopic characterization

The Characterization and Identification of fungal strains were based on their colonial shape, color, spore formation, and the texture of fungal growth. Additionally, the microscopic characteristics of the strain like its conidia or sporangiospores, conidiophores or sporangiophores, and hyphal arrangements were observed under a compound microscope at high power objective (400X). Canon PowerShot A2200 digital camera was used to document the cell and colony morphology of the pesticide-tolerant fungal strain [9, 1].

3. RESULTS AND DISCUSSION

Table 1: Pesticide degrading profile of the selected fungal strains using the DCPIP method

Isolate	Absorbance values (600nm) for pesticide **A**	Percentage degradation (%)	Absorbance values (600nm) for pesticide **B**	Percentage degradation (%)
1	0.583 ± 0.020	11.67	0.401 ± 0.030	41.63
2	0.482 ± 0.010	26.97	0.541 ± 0.050	21.25
3	0.486 ± 0.010	26.36	0.363 ± 0.010	47.16
4	0.595 ± 0.020	09.85	0.514 ± 0.020	25.18
5	0.638 ± 0.060	6.33	0.613 ± 0.060	10.77
6	0.241 ± 0.060	63.48	0.256 ± 0.010	62.74
7	0.267 ± 0.010	59.55	0.289 ± 0.040	57.93
8	0.494 ± 0.020	25.15	0.498 ± 0.030	27.51
Control	0.660 ± 0.011	-	0.687 ± 0.020	-

Table 2: Day 1 colony growth diameter of the isolated strains at different concentrations of pesticide A in ppm

Isolate	**10 ppm**	**50 ppm**	**100 ppm**	**200 ppm**	**500 ppm**
1	2.70 ± 0.47	2.80 ± 0.29	2.20 ± 0.20	2.40 ± 0.42	1.60 ± 0.15
2	1.80 ± 0.11	3.50 ± 0.12	4.60 ± 0.20	2.40 ± 0.44	2.00 ± 0.12
3	1.70 ± 0.47	2.80 ± 0.58	2.00 ± 0.20	2.00 ± 0.10	1.20 ± 0.06
4	1.80 ± 0.00	5.80 ± 0.58	5.80 ± 0.40	3.80 ± 0.50	1.20 ± 0.50
5	2.50 ± 0.50	1.20 ± 0.00	3.50 ± 0.60	2.20 ± 0.20	2.00 ± 0.53
6	1.20 ± 0.29	2.10 ± 1.10	2.50 ± 0.10	2.20 ± 0.20	1.80 ± 0.10
7	1.80 ± 0.00	2.80 ± 0.42	2.50 ± 0.10	3.50 ± 0.12	1.50 ± 0.20
8	1.50 ± 0.50	1.60 ± 0.42	5.60 ± 0.10	3.50 ± 0.05	2.50 ± 0.10

Table 3: Day 1 colony growth diameter of the isolated strains at different concentrations of pesticide B in ppm

Isolate	10 ppm	50 ppm	100 ppm	200 ppm	500 ppm
1	4.60 ± 0.47	4.83 ± 0.29	4.60 ± 0.42	5.13 ± 0.42	4.83 ± 0.15
2	4.90 ± 0.11	4.06 ± 0.12	4.60 ± 0.20	4.50 ± 0.44	4.93 ± 0.12
3	4.60 ± 0.47	5.30 ± 0.58	5.80 ± 0.20	4.93 ± 0.10	3.96 ± 0.06
4	4.80 ± 0.20	5.60 ± 0.58	4.30 ± 0.64	4.30 ± 0.58	5.50 ± 0.50
5	5.50 ± 0.50	5.30 ± 0.58	4.40 ± 0.60	4.90 ± 0.12	4.60 ± 0.53
6	5.84 ± 0.29	4.73 ± 1.10	4.50 ± 0.10	4.40 ± 0.13	3.90 ± 0.10
7	5.00 ± 1.30	5.10 ± 0.42	4.50 ± 0.10	3.90 ± 0.12	4.80 ± 0.20
8	5.50 ± 0.50	5.10 ± 0.42	4.60 ± 0.10	5.00 ± 0.05	4.70 ± 0.10

Table 4: Day 2 colony growth diameter of the isolated strains at different concentrations of pesticide A in ppm

Isolate	10 ppm	50 ppm	100 ppm	200 ppm	500 ppm
1	4.86 ± 0.57	5.03 ± 0.28	4.83 ± 0.40	5.33 ± 0.41	5.10 ± 0.40
2	5.13 ± 0.12	5.53 ± 0.57	4.60 ± 0.20	4.73 ± 0.46	4.20 ± 0.10
3	4.53 ± 0.57	4.02 ± 0.17	5.93 ± 0.12	5.30 ± 0.30	5.60 ± 0.40
4	5.20 ± 1.00	4.60 ± 0.46	4.60 ± 0.76	4.50 ± 0.70	4.60 ± 0.40
5	5.70 ± 0.50	6.23 ± 0.56	5.06 ± 0.14	5.06 ± 0.14	4.80 ± 0.40
6	5.96 ± 0.25	5.53 ± 0.53	2.60 ± 0.60	2.60 ± 0.60	4.00 ± 0.00
7	5.20 ± 1.00	4.93 ± 1.10	4.10 ± 0.10	4.10 ± 0.10	4.80 ± 0.40
8	5.70 ± 0.50	5.30 ± 0.42	5.20 ± 0.10	5.20 ± 0.10	4.70 ± 0.30

Table 5: Day 2 colony growth diameter of the isolated strains at different concentrations of pesticide B in ppm

Isolate	10 ppm	50 ppm	100 ppm	200 ppm	500 ppm
1	5.00 ± 0.00	8.50 ± 0.00	4.40 ± 0.53	5.60 ±0.56	2.90 ± 0.16
2	5.00 ± 0.40	7.16 ± 1.20	6.40 ± 0.40	5.10 ± 0.16	4.30 ± 0.50
3	6.00 ± 0.30	7.30 ± 0.28	3.90 ± 0.40	5.30 ± 0.12	3.10 ± 0.10
4	7.00 ± 0.30	4.83 ± 0.28	4.00 ± 0.18	6.60 ± 0.10	3.10 ± 0.10
5	7.00 ± 0.40	4.30 ± 0.40	4.60 ± 0.21	5.60 ± 0.00	3.40 ± 0.10
6	7.00 ± 0.30	3.00 ± 0.10	3.90 ± 0.16	5.00 ± 0.00	3.00 ± 0.10
7	6.50 ± 0.40	4.20 ± 0.40	3.90 ± 0.46	6.40 ± 0.20	3.00 ± 0.90
8	8.50 ± 0.50	8.50 ± 0.50	8.30 ± 0.76	7.50 ± 0.50	6.50 ± 0.50

Table 6: Day 3 colony growth diameter of the isolated strains at different concentrations of pesticide A in ppm

Isolate	10 ppm	50 ppm	100 ppm	200 ppm	500 ppm
1	4.86 ± 0.57	5.03 ± 0.28	4.83 ± 0.40	5.33 ± 0.41	5.10 ± 0.40
2	5.13 ± 0.12	5.53 ± 0.57	4.60 ± 0.20	4.73 ± 0.46	4.20 ± 0.10
3	4.53 ± 0.57	4.02 ± 0.17	5.93 ± 0.12	5.30 ± 0.30	5.60 ± 0.40
4	5.20 ± 1.00	4.60 ± 0.46	4.60 ± 0.76	4.50 ± 0.70	4.60 ± 0.40
5	5.70 ± 0.50	6.23 ± 0.56	5.06 ± 0.14	5.06 ± 0.14	4.80 ± 0.40
6	5.96 ± 0.25	5.53 ± 0.53	2.60 ± 0.60	2.60 ± 0.60	4.00 ± 0.00
7	5.20 ± 1.00	4.93 ± 1.10	4.10 ± 0.10	4.10 ± 0.10	4.80 ± 0.40
8	5.70 ± 0.50	5.30 ± 0.42	5.20 ± 0.10	5.20 ± 0.10	4.70 ± 0.30

Table 7: Day 3 colony growth diameter of the isolated strains at different concentrations of pesticide B in ppm

Isolate	10 ppm	50 ppm	100 ppm	200 ppm	500 ppm
1	7.00 ± 0.50	5.50 ± 0.50	8.50 ± 0.50	8.83 ± 0.28	6.30 ± 0.58
2	8.00 ± 0.50	8.30 ± 0.20	8.30 ± 0.70	8.60 ± 0.28	8.00 ± 0.50
3	8.00 ± 0.50	6.20 ± 0.53	8.30 ± 0.76	8.83 ± 0.29	8.83 ± 0.29
4	7.50 ± 0.50	6.87 ± 0.12	8.10 ± 1.04	6.50 ± 0.50	8.60 ± 0.29
5	7.50 ± 0.50	6.87 ± 0.12	6.87 ± 0.12	6.50 ± 0.50	8.80 ± 0.28
6	7.80 ± 0.76	7.50 ± 0.50	8.10 ± 0.60	8.00 ± 0.50	7.00 ± 0.28
7	6.75 ± 1.30	7.50 ± 0.50	8.20 ± 0.76	8.50 ± 0.50	4.80 ± 0.28
8	6.50 ± 0.50	7.50 ± 0.50	8.30 ± 0.76	8.50 ± 0.50	8.50 ± 0.50

Table 8: Identification profile of the selected pesticide-degrading fungal strains

Isolate	Macroscopic character	Microscopic character	Tentative identity
1	The colony has a dark surface, flat with a slightly raised center.it is covered with velvety dull gray-green or purplish brown, short-napped mycelium. Reverse is black	The hyphae are septate, dark with lateral and terminal conidiophores of varying size, conidiophores produce long branching chains of brown, smooth-walled, oval, pointed conidia which have dark scars of attachment.	*Cladiosporum* sp.
2	Fast-growing colony. At first, white and cottony but developed red-rose to red color on both sides.	The largest spores are sickle-shaped and may contain several cells. Small spores with one or two cells have more rounded ends.	*Fusarium* sp.
3	Flat, compact colonies, white at first then becoming black, green, bluish, or yellow.	Small one-celled spores irradiating out from the swollen base.	*Aspergillus* sp.
4	Gray to brown to black colony filling a Petri dish in 2 to 3 days. Similar to *Mucor* spp.	Similar to *Mucor* spp. Except for foot-like structures (rhizoids) at the base of spore-bearing hyphae (see arrows). Spores sporangium clear, coenocytic hyphae.	*Rhizopus* sp.

5	Flat, compact colonies, white at first then becoming black, green, bluish, or yellow.	Small one-celled spores irradiating out from the swollen base.	*Aspergillus* sp.
6	White to tan, flat or fluffy, rapid-growing fungus	Note hyphae breaking into arthrospores. Can be confused with Coccidioides immitis.	*Geotrichum* sp.
7	Rapid-growing colonies, grayish to black to brown; underside jet black	Large, hand grenade-shaped spores with both longitudinal and transverse cross walls. Borne singly or in chains. Septate, dematiaceous fungi	*Alternaria* sp.
8	Gray to brown to black colony filling a Petri dish in 2 to 3 days. Similar to *Mucor* spp.	Similar to *Mucor* spp. Except for foot-like structures (rhizoids) at the base of spore-bearing hyphae (see arrows). Spores' sporangium clear, coenocytic hyphae.	*Rhizopus* sp.

DISCUSSION

From the results of the laboratory experiments as shown in Table 8, the fungi species obtained from the soil samples include, *Cladiosporum* sp. *Fusarium* sp. *Aspergillus* sp. *Rhizopus* sp. *Geotrichum* sp. and *Alternaria* sp. The fungi genus was identified based on morphological and microscopic characteristics aided by the Manual of Fungal Atlases. This is similar to the findings of Adelowo et al. [2] who obtained *Aspergillus* sp. and *Fusarium* sp. from soil samples in studying the biodegradable potentials of fungi species on glyphosate. Results in Table 1 showed that Isolate 6 (*Geotrichum* sp.) had the highest degradation potential with 63.48% and 62.74 % on pesticides A and B respectively. However Isolate 5, identified as *Aspergillus* sp., had the lowest degradation potential with 6.33% on pesticide A and 10.77% on pesticide B. This may suggest that *Geotrichum* sp. has enzymes capable of cleaving the carbon-phosphorous bond in glyphosate pesticides. During the evaluation of the pesticide resistance potential of the different isolates, the results in Tables 2-7 reveal that all the isolates exhibited resistance to both pesticides A and B with pesticide B shown to be the most susceptible to fungi resistance in the 3-day study. Results in Table 7 show that isolates 5 (*Aspergillus* sp.) had the highest growth resistance against pesticides A and B at 50 ppm and 500 ppm respectively. Previous studies by Makut and Ibrahim [8], Mohammed and Bartakke [10], and Parte et al., [14] all reveal the presence and biodegradation potential of *Aspergillus flavus* and *Aspergillus niger* on pesticides polluted soil.

4. CONCLUSION

In conclusion, the study has shown that there exists a diverse pool of fungi species in the soil ecosystem which can remediate soil polluted by the excessive use of pesticides in modern commercial farming systems. Enhancing the ability of these indigenous organisms through various

bioremediation technologies will play a key role in keeping the soil ecosystem safe for beneficial microorganisms while ensuring sustainable development.

RECOMMENDATION

It is advised that farmers should be properly guided on the use of pesticides by the appropriate government and - non-governmental organizations to avoid the destruction of useful organisms in the soil which play diverse roles in organic matter decomposition and important biogeological cycles.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals or humans were used for the studies that are based on this research.

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

No author has any conflicts of interest to disclose.

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