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ISOLATION AND IDENTIFICATION OF ASPERGILLUS NIGER FROM PROPANIL-CONTAMINATED RICE FARMS

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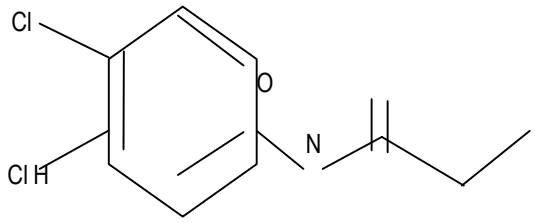
ABSTRACT

The purpose of this study was to isolate Aspergillus niger from Enugu propanyl-contaminated rice farms. A total of eight propanil-contaminated soil samples (two each from Nenwe, Umuueze, Amagunze and Ugbauka) were collected and analyzed for the presence of Aspergillus niger. Aspergillus niger was isolated using Sabouraud dextrose agar (SDA). After the incubation period, the number of unit-forming colonies was counted to determine the average fungal number. Soil samples cultured on Sabouraud dextrose agar plates were found to be overgrown with Aspergillus niger. Aspergillus niger was identified by its macroscopic and microscopic features. The average total fungal counts in the tested soil samples were 3.5 x 102 CFU/mL, 1.8 x 102 CFU/mL, 2.4 x 102 CFU/mL, and 2.8 x 102 CFU/mL for Nenwe, Ugbawka, Amagunze, and Umueze soil samples. was. A total of 7 of the 8 samples (2 from Nenwe, Amagunze, Umuueze and 1 from Ugbauka) were positive for Aspergillus niger, and 8 soil samples yielded atotal of 10 Aspergillus niger isolates. Of the 10 Aspergillus niger isolated, 4 (80%) were found in Nenwe and Amgunze soil samples and 1 (20%) was found in Ugbauka and Umuueze soil samples. Aspergillus niger was more common in the Nenwe and Amagunze soil samples than in the Ugbauka and Umuueze soil samples. The limited number of Aspergillus niger isolated from rice farms may be due to the presence of propanil in soil samples. Therefore, propanil has some effect on the prevalence of Aspergillus niger in soil samples from rice farms.

Keywords: Propanil, Aspergillus miger, Sabouraud dextrose agar, and Rice farm.

INTRODUCTION

Intensive use of herbicides in agriculture is a global problem. Propanil is a post-emergent contact herbicide commonly used for the control of grasses and sedges [1]. This herbicide inhibits the photosynthetic process of broadleaf weeds, causing leaf chlorosis and subsequent necrosis [2,3,4] Propanil is a widely used contact herbicide [5]. With an estimated use of about 8 million pounds in 2016, it is one of the more widely used herbicides in the United States [4] Propanil is said to be in use in approximately 400,000 acres of rice production each year [6]



Propanil is used in southern Brazil to control a variety of grasses, broadleaf and aquatic weeds. Propanil is a contact herbicide used worldwide to control weeds by inhibiting photosynthesis. This compound is widely used in agriculture to control post-emergence weeds in the cultivation of various crops such as rice, wheat, barley, oats and rye. However, propanyl is known to be toxic [7,8]. Several propanyl-degrading microbial strains have been isolated from paddy soils, and most of these studies indicated that 3,4-dichloroaniline is an intermediate in propanyl-degrading [9] Many microorganisms (partly isolated from rice paddies) were selected for their ability to degrade chloraniline.). Mushrooms from natural sources can be studied as powerful tools for biodegradation of toxic organic chemicals [8] Microbial communities in cultivated paddy fields are important for maintaining agroecosystem function. Among paddy organisms, fungi are successful in inhabiting soil due to their high plasticity and adaptive capacity to respond to adverse or adverse conditions [2]. [10,11] reported that fungal diversity and activity are regulated by a variety of organisms (plants and other organisms) and abiotic organisms. (soil pH, moisture, salinity, structure, temperature) factors. About 80,500 species of soil fungi have been identified worldwide [2,4,11] Species of Aspergillus, Penicillium, Talaromyces, Verticillium, Trichoderma, Fusarium, Rhizoctonia, Pythium and Phytophthora are most common in agricultural, horticultural and grassland soil ecosystems [2] Some species of fungi can be classified as plant pathogens, others as biopesticides, and others are essential for the decomposition of organic waste, absorption of heavy metals, and formation of soil structure

[12,13]. Despite improved and more readily available molecular techniques for definitive and acceptable identification of organisms, culture and microscopy remain the primary laboratory tools for fungal detection [14] .Aspergillus is a genus of hundreds of species of fungi found in different climates around the world [15]. Aspergillus spp. are highly aerobic, are found in almost all oxygen-rich environments, and often grow as fungi on the surface of substrates due to the high partial pressure of oxygen [16] In general, they grow on carbon-rich substrates such as monosaccharides (such as glucose) and polysaccharides (such as amylose). Aspergillus spp. This species is a common contaminant of starchy foods (such as gari) and grows in or on many plants and trees [17]. Aspergillus niger is an international representative of microscopic filamentous fungi. The cultivar's primary source is soil, but it is also commonly found in a variety of other sources, including historical and archaeological objects [18,19] or indoor spaces [14]

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STATEMENT OF PROBLEM

In Nigeria, depending on the availability of irrigation water and forage crops, farmers till most of their farms with rice or fallow during the summer months (March to May). It can therefore be assumed that soil microbial prevalence may vary depending on soil conditions (pH, mineral content, etc.) and crop type. Over the past year, public interest in sustainable crop production has increased, with studies of native microbes and analysis of their diversity and activity in soil. However, there are limited scientific reports on Aspergillus niger soils in Nigerian **agricultural paddy fields.**

AIMS AND OBJECTIVE

The purpose of this study was to isolate Aspergillus niger from Enugu propanyl-contaminated rice farms. Specific goals are

1. For the isolation of Aspergillus niger from Enugu paddy fields
2. Measurement of Aspergillus niger infestation in Enugu paddy fields.
3. Measuring the effect of propanyl on microbial presence in agriculture field.,

MATERIALS AND METHODS

MATERIALS

The equipment used for this study included incubator, refrigerator, spatula, Bunsen burner, autoclave, conical flasks, test tubes, wire loop, petri dishes and syringe. The reagents used were lacto phenol cotton blue stain reagents. Media used included Sabouraud dextrose agar (SDA). Other materials included the soil samples from rice farms.

STERILIZATION OF MATERIALS;

All glassware used such as test tubes, beakers, Erlenmeyer flasks The experiments were cleaned with detergent, rinsed with water, air dried and sterilized in a hot air oven at 100 °C. Each material is wrapped in aluminum foil before sterilization.

SAMPLE COLLECTION:

Soil samples were collected from four experimental rice farms (Nenwe, Umueze, Amagunze and Ugbaka), farmers extensively use propanil-containing herbicides to isolate Aspergillus niger. Samples were collected to a depth of 2-5 cm at the entrance and center of each farm using an earth auger, stored in sterile aluminum foil, and transported to the laboratory for microbial counts within 24 hours of collection. Additional materials such as media, reagents and equipment used in this study were provided by the ESUT Applied Microbiology and Brewing Laboratory Department.

MEDIA PREPARATION:

PREPARATION OF SABOURAUD DEXTROSE AGAR

A total of 32.5 g of Sabouraud dextrose agar powder was suspended in 500 ml of distilled water. The medium was dissolved by heating with stirring. It was then autoclaved at 121°C and 15 psi for 15 minutes. The sterilized medium was allowed to cool to approximately 45°C. An antimicrobial agent - chloramphenicol (1 ml) - was then added and mixed thoroughly. The medium was then poured into sterile petri dishes in 15-20 ml portions. The medium was allowed to solidify on the plates before use.

ENUMERATION OF FUNGI FROM SOIL SAMPLES

One gram (1g) of each sample was weighed into an Erlenmeyer flask and 9 ml of distilled water was mixed with the sample. This was placed on a laboratory shaker (S150) for 3 hours to homogenize the solution and used as the stock solution. Ten-fold serial dilutions were performed throughout the homogenized mixture using sterile distilled water as the diluent. Six test tubes containing 9 ml of distilled water were used for serial dilutions. The inoculation used the spread plate technique. Approximately 1 milliliter aliquots of the diluted samples (10^{-3} and 10^{-4}) were spotted onto a Sabouraud dextrose agar plate and spread over the surface of the medium with a sterile cotton swab. Sabouraud dextrose agar plates were incubated at 28°C for 2-10 days and the cultures were examined periodically for fungal growth. Colonies were scored for morphology and purified by repeated subculturing on freshly prepared Sabouraud dextrose agar plates followed by subculturing on sterile Sabouraud dextrose agar slant plates for characterization and identification purposes. We also obtained pure Aspergillusniger colonies as stock cultures

SUBCULTURE OF ISOLATED FUNGI

Single colony growths identified as Aspergillus niger by physical and morphological characteristics were transferred to freshly prepared Sabouraud dextrose agar (SDA) slants.

MORPHOLOGICAL IDENTIFICATION OF ASPERGILLUS NIGER
Aspergillus niger was morphologically identified by its on-plate appearance: color pigmentation, mycelial growth, texture, structure, size and shape.

MICROSCOPIC IDENTIFICATION
Wet mounts for lactophenol cotton blue staining were used to stain and monitor Aspergillus niger isolates.

MICROSCOPIC OBSERVATION OF ISOLATES STAINED WITH LACTOPHENOL COTTON BLUE
Two drops of Lactophenol Cotton Blue Stain were placed on a clean, grease-free slide. A seed wire needle was placed upright on the hottest part of the Bunsen flame just above the blue cone and ignited until the entire length of the wire was red hot. Then cool and place in mushroom broth. A small amount of culture was taken while taking some precautions to avoid dropping the culture on the table. The collected mushroom colony was placed on a clean glass slide, contains 2 drops of Lactophenol Cotton Blue Stain. The neck of the slide burned and the cap was replaced. The material was then very gently pushed out with an inoculation needle. A clean cover slip was then placed on the slide and pressed firmly but gently with a thumb to remove excess dirt. I blotted the excess dirt with blotting paper and let it dry. After allowing the preparation to air dry, the edges of the cover slip were sealed with colorless nail polish. Specimens were examined

using a low power objective, 10x, then 40x magnification to ensure proper observation and identification.

RESULTS : A total of eight propanil-contaminated soil samples (two each from Nenwe, Umuueze, Amagunze and Ugbauka) were collected and analyzed for the presence of Aspergillus niger. Aspergillus niger was more common in the Nenwe soil samples than in the Ugbauka soil samples, according to the study. Morphological and microscopic features of isolated Aspergillus niger are shown in Table 1. The average total fungal counts in the tested soil samples were 3.5 x 10 °cfu/ml, 1.8 x 10 7 cfu/ml, 2.4 x 10 °cfu/ml, and 2.8 x 10 7 cfu in Nenwe, Ugbauka and Amgunze soil samples. /ml was. and umueze (Table.2). Aspergillus niger grew in soil samples grown on Sabouraud dextrose agar, and a total of 7 out of 8 samples were positive for Aspergillus niger (Table 3). Among them are Nenwe, Amagunze, and Umuueze, and one, Ugbauka. A total of 10 Aspergillus niger isolates were obtained from his eight soil samples. Of the 10 Aspergillus niger isolated, 4 (80%) were detected from Nenwe and Amgunze soil samples and 1 (20%) was detected from Ugbauka and Umuueze soil samples (Table 4).

Table 1: Morphological identification of Aspergillus niger from propanil-contaminated field samples

Rice Samples	Soil Morphology	Microscopic examination	Probable Organism
Nenwe	The compact white or yellow basal felt is covered with a dense layer of dark brown to black conidial heads.	The hyphae are septate and hyaline, with radial two-tiered conidial heads	*Aspergillus niger*
Ugbaka	Upper: Black center with white border and compact wool, back center light brown with cream border	Large dark brown conidial heads radiating and divided into columns.	*Aspergillus niger*
Amagunze	Upper: Black center with white border and compact wool, back center light brown with cream border	Large dark brown conidia heads radiating into columns	*Aspergillus niger*
Umueze	The compact white or yellow basal felt is covered with a dense layer of dark brown to black conidial heads.	The hyphae are septate and hyaline, with radial two-tiered conidial heads	*Aspergillus niger*

Table 2: Mean total fungal counts in propanil-contaminated agricultural land samples

Rice soil samples	Mean total counts
Nenwe	$3.5x10^2$
Ugbawka	$1.8x10^2$
Amagunze	$2.4x10^2$
Umueze	$2.8x10^2$

.Table 3: INCIDENCE OF FUNGI FROM PROPANIL CONTAMINATED AGRICULTURAL LAND SAMPLES

Sample	Number of Samples	No of occurence	% Occurrence
Nenwe	2	2	100
Ugbawka	2	1	50
Amagunze	2	2	100
Umueze	2	2	100
Total	8	7	87.5

Table 4: FREQUENCY OF *ASPERGILLUS NIGER* FROM PROPANIL CONTAMINATED AGRICULTURAL LAND SAMPLES

Organisms	Occurrence	% Occurrence
Nenwe	4	40
Ugbawka	1	10
Amagunze	4	40
Umueze	1	10
Total	10	100

DISCUSSION

The results of this study showed that Aspergillus niger was present in soil samples from rice farms. The physicochemical properties of the collected soils were not investigated in this study. samples were contaminated with Aspergillus niger (87.5%), which is consistent with other studies [20,5.21] A total of eight soil samples taken from Nenwe, Umuueze, Amagunze and Ugbauka rice farms were analyzed for the presence of Aspergillus niger. Table 2 shows the average total fungal counts in soil samples from farmlands in Nenwe, Umuueze, Amagunze and Ugbauka. The fungal counts in Nenwe, Ugbauka, Amagunze and Umuueze soil samples were 3.5 x 102 CFU/mL, 1.8 x 102 CFU/mL, 2.4 x 102 CFU/mL and 2.8 x 102 CFU/mL. The difference in this number can be attributed to soil type and farmland conditions. Fields from which soil samples were collected and media were used [22] [23] Sabouraud Dextrose Agar (SDA) was used for this study. [24] reported that Sabouraud Dextrose Agar (SDA) medium is optimal for studying mycelium growth with nutrient feeding. A total of 7 out of 8 samples were positive for Aspergillus niger (Table 3). Among them are two of him in Nenwe, Amagunze and Umuueze and one of him in Ugbauka. A total of 10 Aspergillus niger isolates were obtained from 8 soil samples. Of the 10 Aspergillus niger isolated, 4 (80%) were detected from Nenwe and Amgunze soil samples and 1 (20%) was detected from Ugbauka and Umuueze soil samples (Table 4). Suleiman and Akaajimwe (2015) also found that the higher numbers of fungi were isolated from the lowland cultivated soil than the upland cultivated soil and the Aspergillus species were dominant in the soil fungal community. All the Aspergillus niger isolates from agricultural soil samples from Nenwe, Ugbawka, Amagunze and Umueze used in this study point out to the extreme adaptability of this species across a wide pH range and its high tolerance to metal contamination, despite changes in the macro- and microstructure. Among the different type of soil samples, Aspergillus niger strains were more prevalent in Nenwe and Amagunze soil samples than the Ugbawka and Umueze soil samples, all of which were contaminated with heavy metals and toxic elements exceeding the limit values for soils because of the sloppiness of the soil or point of collection of the soil samples from each rice farms. This contamination is in most cases caused by use of herbicides on the soils[24]. Aspergillus niger in the soil play a major role in the decomposition of rice straw in compost by producing extracellular enzymes like cellulase (TC), endo-beta-1,4 glucanase (EG), and endo-beta-1,4 xylanase (XYL) [15]. In addition to Aspergillus, all types of fungi play an important role in nutrient utilization. Aspergillus niger can also aid in phosphate dissolution by increasing the bioavailability of phosphorus in plant soils [25]. The presence of Aspergillus is a good indicator of nutrient utilization in the soil. Previous reports indicate that tropical Asian rice is predominantly composed of Aspergillus species [19] which grow at 25–30 °C in paddy fields [25], 25–35 °C in white ground and was contaminated by Rice grows rice and brown rice [25]. As for fungal infections associated with rice-growing areas, the data showed that fungal contamination varied widely across the rice-growing chain. This is because current traditional agricultural practices provide favorable conditions for

fungal growth [26]. Nenwe, Umueze, Amagunze, and Ugbauka is contaminated with propanil due to rapid and continuous use of herbicides and fertilizers. The reason for the limited numbers of Aspergillus niger isolated from agricultural rice fields is the presence of propanil in soil samples and the use of inorganic herbicides with low levels of nitrogen and organic carbon that reduce microbial communities. This may be because ,especially the number of Aspergillus niger in the soil. Some herbicides appear to specifically affect the nitrogen-fixing capacity of microbes, with inhibition observed in nitrogen-free media, but not in the presence of inorganic substances.

CONCLUSION

From the results of this study, it can be concluded that Aspergillus niger is present in the rice farms of Nenwe, Umueze, Amgunze and Ugbauka in Enugu province. Soil samples from Nenwe, Umueze, Amagunze and Ugbauka are believed to be contaminated with propanil due to overuse of herbicides and fertilizers. The limited numbers of Aspergillus niger isolated from rice farms may be due to the presence of propanil in soil samples. Therefore, propanil has some effect on the prevalence of Aspergillus niger in soil samples from rice farms.

RECOMMENDATIONS

Based on the results of this study, the following recommendations were made: o Molecular identification of Aspergillus niger isolated from paddy soil is needed for further work. o To reduce harmful chemicals (such as propanil) from the environment, the combination of biological agents (bacteria, fungi) and innovative molecular technologies must be beneficial to their respective effects .Others are applicable to wider land/water areas. o Further research on the prevalence of other fungi on agricultural land should be carried out. is also checked. o Knowledge of Aspergillus niger activity in soil needs to be investigated. o Studies on the microbial degradation of pesticides and their effects on the microflora and microbial activity in rice soils that have been mainly flooded so far is limited to short-term laboratory testing and should be conducted according to the following: More realistic field conditions and long-term cultural practices.

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