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Molecular identification and characterization of *Aspergillus Species* isolated from dumpsites in Federal University of Technology, Owerri (FUTO)

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ABSTRACT: ***Aim:*** *To identify and characterize Aspergillus species isolated from dumpsites in Federal University of Technology Owerri (FUTO)* ***Study Design****: This was a simple randomized sampling study technique.* ***Methodology****: Three separate dumps on the school premises, soil samples were taken from three different dumpsites: the Girls' Hostel, the Ihiagwa, and the Eziobodo. We assessed the soil's physicochemical properties by letting it air dry and then filtering it through a 2 mm screen. For this purpose, we measured the soil's texture using a Bouyoucos hydrometer. A pH meter was used to test the pH of a soil-water extract with a ratio of 1:2.5. An electrical conductivity meter was used to measure the electrical conductivity, organic carbon, and Walkley and Black. The wet oxidation technique was used to determine the organic carbon content. Total nitrogen is evaluated using the micro Kjeldahl procedure, whereas accessible phosphorus is assessed using the Bray and Kurtz technique, as stated by Horneck and Miller (20). The discovery of exchangeable bases followed the use of ammonium acetate for extraction. Fermimeter readings for potassium (K) and calcium (Ca) and magnesium (Mg) were taken at the Soil Science Laboratory using the EDTA titration method, respectively. Potato Dextrose Agar (PDA) was used to separate the Aspergillus species from the samples. Molecular techniques, such as ITS, were used for a more comprehensive identification after the organism had been identified using macroscopy and microscopy. We used Graphpad Prism version 8 to examine the data.* ***Results****: Two landfill locations were found to have Aspergillus niger. Aspergillus flavus was found in three different dumpsites: the girls' hostel dumpsite, the Ihiagwa dumpsite, and the Eziobodo dumpsite. Aspergillus aculeatus was found in three different dumpsites: the girls' hostel dumpsite, the Ihiagwa dumpsite, and the Eziobodo dumpsite. The dumpsites of the girls' hostel at Ihiagwa and Eziobodo were found to have Aspergillus fumigatus in addition to Aspergillus flavipes. Molecular analysis confirmed the presence of Aspergillus niger and Aspergillus aculeatus. In contrast to the controlled site's mean pH value of 39.8±0.08, the pH values of the soil samples taken from the investigated dumpsites varied between 6.38±0.10 and 7.11±0.10. Based on the data we have, the dumpsites that were collected had the following pH, Cation Exchange Capacity (cmol+kgG1), Calcium (mEq/ 100 g), Potassium (mEq/ 100 g), 0.59, 0.48, 0.79, and Nitrogen (mEq/ 100 g) values: 0.98, 1.84, and 0.73, respectively.* ***Conclusion****: The investigation indicated that all areas investigated had Aspergillus species. The study included the use of molecular and culture-based approaches to identify and isolate fungi. Aspergillus aculeatus and Aspergillus niger were identified using molecular methods in this investigation. Microbes abound in contaminated soils; some of them may be beneficial to humans, while others may pose health risks. This study discovered that soil from dumpsites contains several dangerous species of the Aspergillus fungus. To better inform the public about the dangers of living near dumpsites, this study will shed light on the kinds of fungus that thrive in the soil there.*

Keywords*: Dumpsite, Aspergillus Niger,* Molecular, *Physiochemical Properties, Federal University of Technology (FUTO)*

INTRODUCTION

Soils naturally occur throughout the majority of Earth's surface and mediate interactions between the three phases of matter: gases, liquids, and solids, which may be either living organisms or inert geological formations. According to Patterson et al. (2010), the majority of soil consists of organic, inorganic, liquid, and gaseous components. Soil is multi-faceted, including not just its chemical (such as pH), physical (such as texture), and biological (such as biodiversity) characteristics, but also three additional elements [1].

"Dumpsite" is shorthand for a certain kind of landfill [2]. There are usually no environmental controls at landfills as most people just dump their rubbish wherever they like. Consequently, there may be periods when certain areas along main roadways have a concentration of solid garbage. There have been worries about environmental health, including respiratory problems and malaria, linked to open dumps in residential areas [3]. According to [4], infections may arise as a result of inoculums of pathogens that are

discharged into the soil at dumpsites. Fungal infections are the most harmful soilborne pathogens [5]. They are the most common cause of root rots, wilts, blight, and other plant diseases that are often difficult to cure with standard techniques. Species of Fusarium, Rhizoctonia, Verticillium, Sclerotinia, Ralstonia, Pectobacterium, and Streptomyces are among the many soilborne pathogens that may adversely affect plants.

Identifying fungal species using morphological and microscopic methods is time-consuming and error-prone [6]. While conventional cultural methods have long relied on microscopic features and an organism's shape, molecular characterisation, which is based on its genetic makeup, provides a wealth of new information. Fungal species may be determined when the Internal Transcribed Spacer (ITS) region of the fungus is sequenced and amplified using Polymerase Chain Reaction (PCR). Since molecular approaches may identify species that have never been seen before, they should be used in microbial diversity investigations [7].

The variety of soil organisms may be better understood and countermeasures can be devised if the soil microbial community is characterised. For the most part, researchers looking at Nigerian microbes have used soil samples taken from landfills as a starting point for their identification efforts. In order to find the best taxonomy approach, this research used both traditional culture methods and basic molecular techniques to isolate and identify the Aspergillus species linked to the Federal University of Technology, Owerri (FUTO) dump site. The culture approach was used to isolate the organisms, since the molecules were mainly utilised for species identification in the research.

Figure 1: Map of the Study Area

= Sample titre value
= Blank titre value.

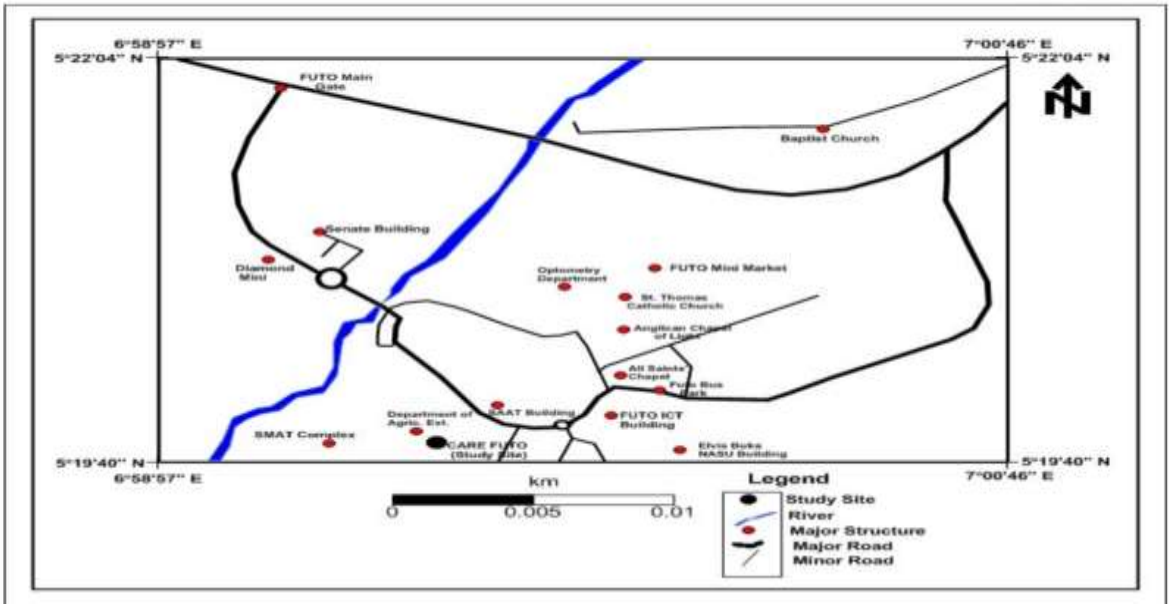

Fig. 1. Map of Federal University of Technology Owerri (FUTO)

Warrior State, Owerri is the capital of Imo State in Nigeria, and it is also the site of the government financed Federal University of Technology Owerri (FUTO). The university's campus is surrounded by the settlements of Eziobodo, Ihiagwa, Obinze, Okolochi, and Emeabiam. It is the premier federal university of technology in the southeast and southwest areas of Nigeria.

With the formation of the first provisional council and the appointment of its members, Shehu Shagari, the first executive president of Nigeria, established the institution in 1980 by executive fiat. This is the first technology university in the nation. This particular state does not have a conventional university; therefore, it became the first of three such schools as part of the Nigerian federal government's plan to establish a University of Technology in every geographic zone. The open dumpsite at Federal University of Technology Owerri (FUTO) was used for the disposal of various biodegradable materials, including papers, bottles, food scraps, plastics, tin cans, grasses, polyethene bags, leather, and more.

COLLECTION OF SOIL SAMPLE

The school's three dumpsites—the Girls' hostel dumpsite, the Ihiagwa dumpsite, and the Eziobodo dumpsite—were each sampled for soil. Using an auger borer, samples were gathered at a depth of 0-15 cm from beneath the garbage pile. 15 milligrammes of dirt was gathered. Before being sent to the lab for fungal isolation, the dirt was placed in a sterile zip bag. Part of each soil sample from each of the three locations was submitted to the microbiology lab at Science Laboratory Technology (SLT) to isolate a pure culture of Aspergillus specie, while the other half was used to analyse the soil's physiochemical parameters.

ANALYSIS OF SOIL PHYSIOCHEMICAL PROPERTIES

The soil was air dried and then filtered through a 2 mm mesh screen to ascertain its physicochemical properties. Using the Bouyoucos hydrometer approach, the soil texture was evaluated [8]. Wet oxidation was used to determine organic carbon, pH was determined by measuring the electrical conductivity (EC) of a soil-water extract, and the findings were compared to those of Walkley and Black [9]. The Bray and Kurtz method was used to find the available phosphorus, whereas the micro Kjeldahl method was used to get the total nitrogen, as described by Horneck and Miller (20). Finding the bases that may be exchanged when extracting ammonium acetate. Potassium (K), calcium (Ca), and magnesium (Mg) were measured at FUTO's Soil Science Laboratory using flamephotometers, EDTA titration, and other methods.

ISOLATION AND MORPHOLOGICAL IDENTIFICATION OF *ASPERGILLUS* ISOLATES:

We homogenised three separate soil samples collected from various trash sites as part of the dilution plating method (24). After that, we seeded 1 mL of a combination consisting of 1 g of soil sample and 9 mL of sterile water into each figure of Potato Dextrose Agar (PDA). For the incubation process, the plates were kept at room temperature. next the first 48 hours, data was examined daily for the next seven days. All fungi that were suspected of being Aspergillus species were phenotypically identified using agar (PDA) plates. Based on published guidelines and observations made under a light microscope with 10 and 40× objective lenses, isolates were identified by observing the colour and type of fungal growth on the surface and back side of the growth medium, as well as by observing the nature of the hyphae, the presence or absence of spores, and other microscopic structures. The total number of colonies at each location was recorded with great care. By summing together each site's particular Aspergillus abundance, we were able to determine the overall CFU gG1. To measure the species variety in all places, Shannon-Wiener's index (H“) was used after Keylock's description. Aspergillus Niger and Aspergillus aculeatus specimens were supplied for molecular confirmation.

MOLECULAR IDENTIFICATION

DNA EXTRACTION

This extraction was carried out using the ZR fungal/bacterial DNA micro prep extraction kit that was supplied by Inqaba South Africa. A 200 εL isotonic buffer was used to suspend the ZR Bashing Bead Lysis tube, which contained a strongly developed pure culture of the isolates under suspicion. Then, 750 µL of lysis solution was added to the tube. The tubes were subjected to a fast treatment for 5 minutes using a bead beater that was equipped with a 2ml tube holder assembly. There was a one-minute period of 10,000xg spinning of the ZR bashingbead lysis tube.

Centrifuged at 7000 xg for 1 minute, the orange-topped Zymo-Spin IV spin Filter was used after 400 microlitres of supernatant had been transferred to a collection tube. In a different collecting tube, 800 microlitres of the filtrate was moved to a Zymo-Spin IIC column prior to centrifugation at 10,000xg for 1 minute. The filtrate in the collecting tubes was mixed with 1,200 mL of fungal/bacterial DNA binding buffer to make a final volume of 1600 mL. After then, the column had its flow through eliminated. This Zymo-spin also spun the remaining chapters of the book. The Zymo-spin IIC was mixed with 200 εL of DNA Pre-Was buffer in a different collection tube. Then, for one minute, the mixture was spun in a centrifuge at 10,000 x g. A fungal/bacterial DNA wash buffer, volume 500 µL, was then added.

A fresh 1.5 mL centrifuge tube was used to transfer the Zymo-spin IIC column. The column matrix was then supplemented with 100 mL of DNA elution buffer. Following this, the DNA was extracted by centrifuging the mixture at 10,000 xg microlitres for 30 seconds. After that, the ultra-pure DNA was stored at -20 degrees until it was needed for more reactions.

DNA QUANTIFICATION

We quantified the extracted genomic DNA using the Nanodrop 1000 spectrophotometer. You may open the Nanodrop app by double-clicking its icon. A two-ounce volume of sterile distilled water was used to initialise the equipment after its blanking with normal saline. A lower pedestal was used to touch the two microlitres of extracted DNA, and the upper pedestal was lowered to do the same. To find the DNA concentration, just click the "measure" button.

INTERNAL TRANSCRIBED SPACER (ITS) AMPLIFICATION

Using a 30 microlitre final volume and 35 cycles on an ABI 9700 Applied Biosystems thermal cycler, the ITS region of the isolates was amplified with the ITS1F: 5'-CTTGGTCATTTAGAGGAAGTAA-3' and ITS4: 5'- TCCTCCGCTTATTGATATGC-3 primers. The following components were added in the PCR mix: primers at a concentration of 0.4M, the extracted DNA as a template, and the X2 Dream Taq Master mix, which was provided by Inqaba, South Africa: Taq polymerase, DNTPs, and MgCl. These were the PCR parameters: initial denaturation at 95ºC for 5 minutes, denaturation at 95ºC for 30 seconds, annealing at 53ºC for 30 seconds, extension at 72ºC for 30 seconds for 35 cycles, and final extension at 72ºC for 5 minutes. After 15 minutes of resolution on a 1% agarose gel at 120V, the result was seen using a blue light transilluminator.

SEQUENCING

Inqaba Biotechnological of Pretoria, South Africa, used a 3510 ABI sequencer and the BigDye Terminator kit to do the sequencing. With a final volume of 10 ul, the following components were used for the sequencing: 0.25 ul of BigDye® terminator v1.1/v3.1, 2.25 ul of 5 x BigDye sequencing buffer, 10uM of primer PCR, and 2-10ng of PCR template per 100 bp. The following conditions were used for sequencing: The temperature will be varied in 32 cycles of 96°C for 10 seconds, 55°C for 5 seconds, and 60°C for 4 minutes.

PHYLOGENETIC ANALYSIS

In order to alter the obtained sequences, the bioinformatics program Trace edit was used. Similarly, comparable sequences were retrieved from the NCBI database using BLASTN. We used ClustalX to align these sequences. Using MEGA 6.0's Neighbor-Joining approach, we were able to deduce the evolutionary history [10]. The evolutionary history of the species that were studied is represented by the bootstrap consensus tree that is estimated from 500 replicates [11]. Using the Jukes-Cantor technique, the evolutionary distances were determined [12].

RESULTS

SOIL PHYSICOCHEMICAL

In Table 1 we can see the locations' physical and chemical properties. Soil salinity is not an issue since the pH is neutral (6.37-8.11) and the electrical conductivity is low. Soil organic carbon levels were low, ranging from 0.44% to 0.50%. Near the ladies' hostel and the Eziobodo waste, the levels of N, P, K, and CEC were higher.

Table 1: Physical and chemical characteristics of soil from experimental sites at 0-15 cm depth

SOIL/DUMPSITE LOCATION	GIRLS HOSTEL DUMP SITE	EZIOBODO DUMP SITE	UMUCHIMA DUMP SITE	CONTROL
pH (1:2.5)	6.81± 0.10	6.38±0.10	7.11±0.10	3.98 ±0.08
EC (dS mG1)	0.40±0.02	0.43±0.00	0.63±0.01	0.18. ±0.02
OC (%)	0.44±0.03	0.48± 0.00	0.50± 0.01	0.20 ± 0.03
Texture				
Sandy	62.5± 0.1	65.8± 0.02	68.3± 0.1	84.32 ± 0.13
Clay	15.8± 0.02	16.2± 0.05	15.4± 0.03	11.50 ± 0.05
Slit	21.7± 0.10	18± 0.02	16.3± 0.08	1.24 ± 0.10
N (mEq/ 100 g)	0.98 ± 0.00	1.84 ± 0.00	0.73 ± 0.00	0.03 ± 0.00
K (mEq/ 100 g)	0.59± 0.03	0.48± 0.01	0.79± 0.03	0.02 ± 0.01
Ca (mEq/ 100 g)	4.50 ± 0.08	4.24± 0.22	4.76 ± 0.00	1.29 ± 0.10
Mg (mEq/ 100g)	1.45±0.00	0.22±0.10	2.91±0.10	0.30 ±0.09
CEC (cmol+kgG1)	13.19±0.00	11.22±0.03	13.32±0.01	20.15±0.03

EC:Electrical conductivity, OC: Organic carbon, N:Nitrogen, P: Phosphorus, K: Potassium, Ca: Calcium, Mg: Magnesium andCEC: Cation exchange capacity

FUNGAL ISOLATES OBTAINED FROM DUMPSITE SOIL

Five (5) Aspergillus fungus were identified from three (3) soil samples taken from the dumpsite. The fungal pure cultures are shown in Fig. 1. The frequency of each isolate was determined. Isolation of Aspergillus niger, Aspergillus flavus, and Aspergillus fumigatus, as well as Aspergillus flovigipes and Aspergillus aculeatus, was carried out at three (3) trash sites and two (2) disposal sites, respectively. Following seven days on potato dextrose agar medium, the physical traits and occurrence rates of the isolates are shown in Table 2.

Table 2. Fungi isolated from dumpsite soil at room temperature (25°C ± 2°C)

Dump site	Morphology	Frequency of Occurrence	Suspected Organism
These dumpsites are located in the girls' hostel, Ihiagwa, and Eziobodo.	At first, the growth was white, but after a few days, it became black.	3	*Aspergillusniger*
Girls' hostel dumpsite, Ihiagwa dumpsite and Eziobodo dumpsite.	Colonies that changed colour from olive green to yellowish green or even black ultimately covered with conidia. The colonies were encircled by a white circle.	3	*Aspergillus flavus*
Girls' hostel dumpsite, Ihiagwa	Produced dark brown to black conidia	2	*Aspergillus aculeatus.*
Ihiagwa dumpsite and Eziobodo dumpsite	Produced cotton-like and velvety colonies	2	*Aspergillus flavipes*
Ihiagwa dumpsite and Eziobodo dumpsite	Colonies were typically blue-green with a suede-like surface consisting of a dense felt of conidiophores	2	*Aspergillus fumigatus*

CONFIRMATION OF ITS GENE OF FUNGAL ISOLATES

The represented sample's fungal-specific ITS gene region was effectively identified by conventional PCR. The Fungi DNA was proven to be present in the samples by the amplification of the targeted ITS region, which validates the specificity of the PCR technique for Fungi identification. Just like the picture down below.

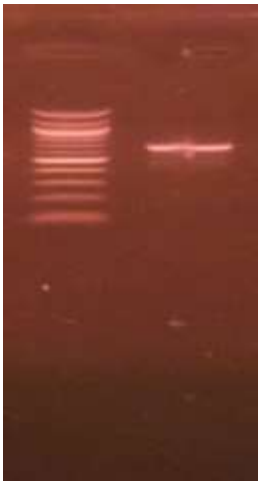

Fig 1: Agarose gel electrophoresis showing the amplified ITS. Lane 1 represents the amplified 650bp while lane L represents the 100bp DNA ladder.

The obtained ITS sequence from the isolates produced an exact match during the megablast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) database. The ITS of the isolates showed a percentage similarity to other species at 100%. The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement of the ITS of the isolates within the *Aspergillus*sp and revealed a closely relatedness to *Aspergillus aculeatus.*

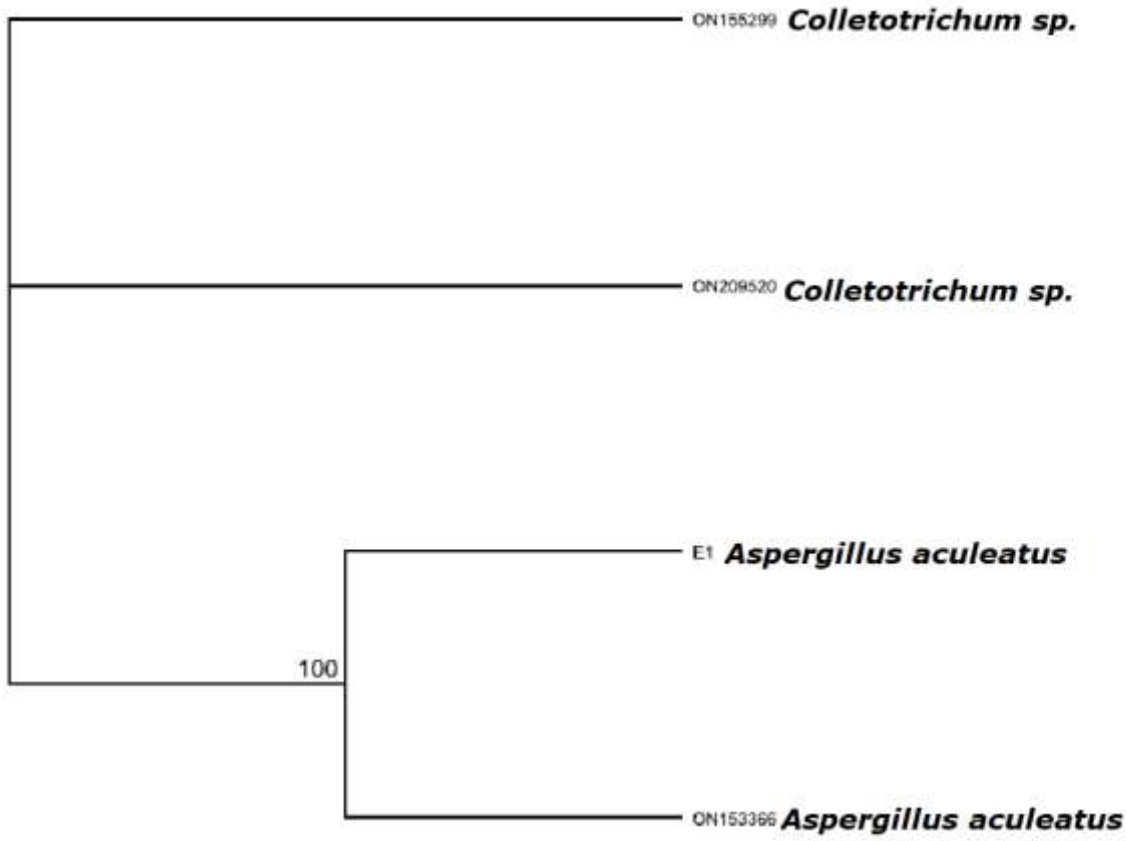

Fig 2. Phylogenetic tree showing the evolutionary distance between *Aspergillus aculeatus* isolates from Dumb sites.

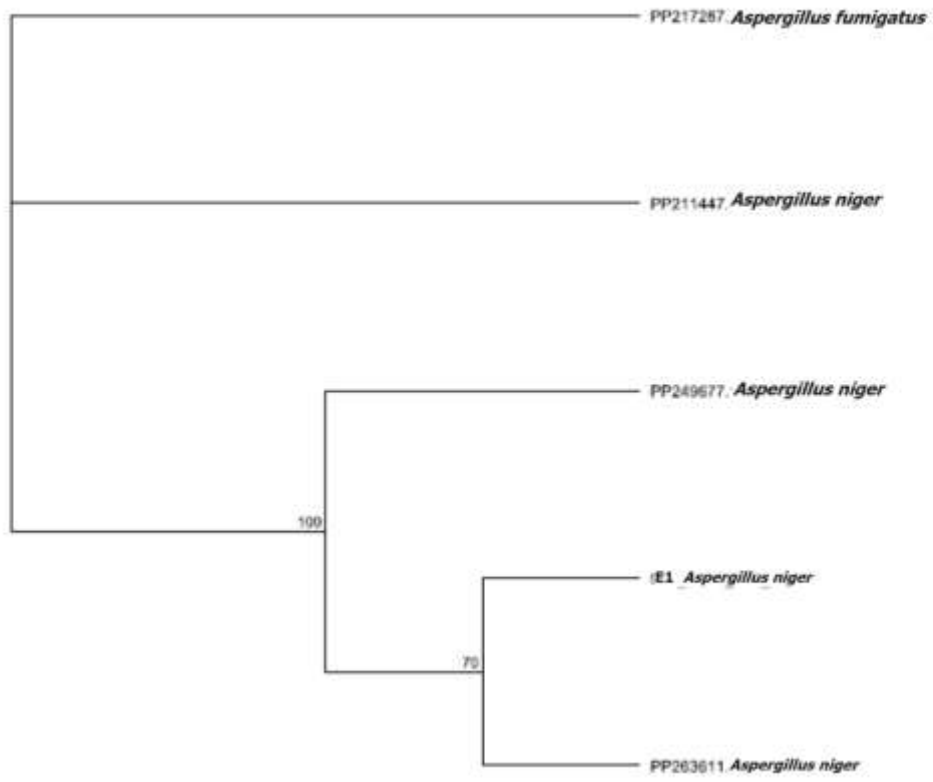

Fig 3: Phylogenetic tree showing the evolutionary distance between *Aspergillus aculeatus* isolates from Dumb sites.

DISCUSSION

Particularly in agricultural, forest, and waste soil, fungi play an important role in the soil microbiota and are necessary for the proper functioning of soil ecosystems. Phosphate solubilisation and possible complicated chemical compound breakdown are two important roles played by the Aspergillus fungus in the essential nutrition cycle [13]. Every single landfill tested positive for Aspergillus species, according to the research. However, research revealed that various land uses and fertiliser treatments affected their diversity and composition. Surprisingly, Aspergillus has been discovered to be prevalent in almost all ecosystems [14]. They have the ability to bounce back and adjust to different challenging conditions. The ITS1-2 gene sequences of the fungal isolates were amplified using polymerase chain reaction, yielding amplicons ranging from 580 to 800 base pairs. Research on biodiversity has shown that when compared to more conventional cultural approaches, the Internal Transcribed Spacer (ITS) region provides a more precise way to distinguish between various species of fungi [15]. This investigation's molecular techniques were effective since two representative fungal species, Aspergillus niger and Aspergillus aculeatus, were successfully identified.

The prevalence of Aspergillus niger and Aspergillus flavus in all areas was determined by Peronne et al., who shared our findings. According to [16], a wide variety of aspergillus species were detected in agricultural soils. Some of them were nigri, flavus, parasiticus, ochraceus, carbonarius, and alliaceus. Similar organisms were found in soil and corn at alarmingly high rates in previous studies conducted in Kenya [17] and Nigeria [18]. Fungal species comparable to those recovered in this study were found by other researchers using the conventional culture method of characterisation. The most frequent species in the waste dumpsites studied were Aspergillus. Among the many bacterial and fungal species discovered by Simon-Oke and Alade during their investigation of two dumpsites in the Nigerian city of Akure were Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, and Mucor mucedo [19]. The scientists hypothesised that the small size of one of the dumpsites and the relative lack of human activity in the region may be to blame for the low bacterial and fungal counts. The following species of fungi were identified as having their origins in dumpsites in Bwari, Federal Capital Territory, Nigeria: Aspergillus niger, Fusarium spp., Rhizopus spp., and Mucor spp. [20]. In their

investigation of Port Harcourt dumpsites, Williams and Hakam identified many species of Aspergillus, Fusarium, Mucor, Penicillium, and Saccharomyces [21].

Molecular characterisation in this study revealed the presence of additional species such as Aspergillus flavipes, Aspergillus stemplicola, Trichoderma harzianum, and Cunninghamellabinariae in dumpsite soil, even though the same species were also discovered in the soil using the traditional cultural method. Soil microbial communities are known to be more diverse and abundant during wetter seasons [19]. All of the fungal isolates were effectively described to the species level thanks to the application of molecular methods in this investigation.

Some of the fungal species associated with dumpsite soil have been discussed in this research so that readers may have a better understanding of the possible benefits and drawbacks of the isolates. There has also been an emphasis on the isolated' economic importance. The organisms obtained during this inquiry include both plant pathogens and those that are harmful to public health. In a first for the Federal University of Technology, Owerri (FUTO), researchers used macroscopic features of colonies and microscopic traits from a small number of waste sites to identify five major species of Aspergillus.

CONCLUSION

All of the dumpsites that were tested for Aspergillus species. Every single one of the dumps has Aspergillus niger and Aspergillus flavus. The dumpsites at Ihiagwa and the Girls' Hostel were found to contain Aspergillus aculeatus, whereas Eziobodo was found to have Aspergillus fumigatus and Ihiagwa aspergillus flavipes, respectively. The study included the use of molecular and culture-based approaches to identify and isolate fungi. Two species of Aspergillus, A. aculeatus and N. niger, were identified in this research by molecular analysis. Despite the exclusion of other genera and species that are economically significant, the study only addresses Aspergillus species. Many different kinds of bacteria, some of which may be helpful and others of which might be harmful, find a suitable home in garbage dumps. Soil samples taken from landfills were found to contain several species of the medicinal fungus Aspergillus, according to the study's findings. The current study's findings will aid in public education on the risks of living near dumpsites and add to our understanding of the fungal species linked to dumpsite soil.

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