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Isolation and morphological identification of fungal entomopathogens from soil samples and cockroaches

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Abstract
Chemical insecticides have been utilized to reduce insects as vectors of diseases which cause adverse effect to the environment and non-target organisms. Entomopathogenic fungi contain bioactive compounds that kills target insect from larvae to adult stage. This research investigated the morphological identification of entomopathogenic fungi from soil samples and cockroaches (*Periplaneta Americana).* Fungal entomopathogens were isolated from soil samples and cockroaches (*Periplaneta Americana*) through soil suspension and selective media procedures. Fungi isolates were subjected to macro and micro morphological identification. Three fungal entomopathogens were successfully isolated and identified from soil (D1, D2, D3) namely *Geotrichum*, *Penicillium* and *Aspergillus* species. Entomopathogens isolated from cockroaches C1, C2 were of the genus *Aspergillus* and C3 was identified as *Penicillium* sp. Fungi isolates obtained from soil samples and cockroaches belong to the genus *Geotrichum, Penicillium* and *Aspergillus* respectively.

Keywords: Bioinsecticide, entomopathogenic fungi, *Aspergillus, Penicillium, Geotrichum*

1. Introduction
The most widely used control measures for the prevention of mosquito related diseases includes proper sanitary measures, habitat destruction and protecting oneself from mosquito bites [1] Many countries over the years arranged official programs for the control of mosquitoes [2]. At this moment, synthetic insecticides against adults or larvae have been the cornerstone and popular method employed for control of mosquitoes [3]. Mosquitos' larvae are sensitive targets for these insecticides because mosquitoes breed in water which makes it easy to deal with them in their natural habitat [4, 5]. It is crucial to investigate the potentials of natural materials that are not only efficient in combating insects but also safe for health, environment and non-target organisms leading to the application fungal entomopathogens as biocontrol agents.

Fungal entomopathogens known for their larvicidal activity are *Geotrichum* sp, *Beauveria* sp, *Metarhizium* sp, *Lecanicillium* sp, *Hirsutella* sp and so on. These fungi are known to produce destruxin A, B, C, Kojic acid, beauvaricin, bassianin and hirsutellin compounds which have insecticidal activity [6]. These compounds contribute immensely to the infection process in target organisms such as insects, beginning from conidial attachment, penetrating the host's body and destroying its digestive tract [7]. The spores produced by entomopathogens are mainly adapted for both dispersal and infection due to the complex nature of the insect cuticle, these spores possess special properties enabling their attachment and penetration to the cuticle. [6, 7]. The spores attach to the cuticle taking advantage of the mucilaginous matrix surrounding them and also combination of electrostatic forces. In addition, chemical agents such as lipoproteins which facilitate attachment to the epicuticle; forming attachment-enhancing structures called appressoria [7]. The types of fungi that act as potential biocontrol agents for insect disease vectors are still limited, therefore, this research investigated the isolation and morphological identification of entomopathogens originating from soil samples and cockroaches.

2. Materials and Methods

The entire study was carried out in July- December, 2022 at the department of Microbiology, Faculty of Life Sciences, Bayero University, Kano, Nigeria. The equipment and materials used for this study were of analytical grade.

2.1 Soil sample collection and storage

Soil sample was collected from three different locations around Biochemistry department, Bayero University Kano (11.9836ºN 8.4753ºE). The soil surface was first cleared of leaves and other litters and a hole 10cm below the ground was made using hand-trowel. About 200g of soil was then collected in a clean polyethene bag. The soil collected was air-dried and then stored at room temperature [8].

2.2 Morphological Identification of Entomopathogenic Fungi

Isolation of fungi was carried out in microbiology department Bayero University, Kano, Nigeria using soil suspension and selective media procedures. Soil samples collected and properly sieved for reducing soil lumps [9]. Serial dilutions were made and from each dilution was plated on DOA (dodine oatmeal agar) selective medium for screening entomopathogenic fungi. Insects used for the isolation of the entomopathogens were cockroaches. This insect was left to die and transferred to a petri dish covered with damp sterile filter paper. The petri dishes were left at room temperature (28-30 ºC), for 10 days until the body of the insect exhibited fungal growth. The fungi which grow on the insect body were isolated, then plated on the PDA medium and incubated at room temperature (28-30 °C) for 14 days [10]. The results of morphological features are explained in table 1 and 2.

3. Results

The results showed that 6 fungi isolates were gotten from soil samples and cockroaches and identified using morphological features.

Table 1: Morphological characteristics of the colony of fungi isolates from soil samples and cockroaches

Numbers	Soil Samples/Cockroaches	Codes for Isolates	Colour		Nature	Edges
			Surface	Base		
I.	Soil samples	D1	Gray	Glassy	Spherical	Circular
II.		D2	Dark Green	Glassy	Spherical	Undulate
III.		D3	Yellowish	Glassy	Spherical	Thread-like
IV.	*Periplaneta americana*	C1	Green	Transparent	Spherical	Undulate
V.		C2	Black	White	Spherical	Undulate
VI.		C3	White	Amorphous	Myceloid	Thread-like

After the macroscopic features was observed, then identification of entomopathogens was further investigated using microscopic characters. Macroscopic features of Isolate D1 (Table 1) comprises gray colony and cotton-like filaments characterized by smooth edges. Isolate D2 consist of insulated hyphae with absence of conidiophores.

Table 2: Microscopic identification of entomopathogens from Soil Samples and cockroaches

Codes for Isolates	Structure of conidia cell	Conidiophores	Hypha	Vesicles	Phialide	Foot-cell	Identification
D1	Cylindrical	Absent	Insulated	Absent	Absent	Not found	*Geotrichum specie*
D2	Globular	Branched	Insulated	Absent	single	Not found	*Penicillium specie*
D3	Globular	Upright	Insulated	Round	single	Yes	*Aspergillus specie*
C1	Globular	Upright	Insulated	Round	single	Yes	*Aspergillus specie*
C2	Globular	Upright	Insulated	Round	single	Yes	*Aspergillus specie*
C3	Globular	Branched	Insulated	Round	single	Not found	*Penicillium specie*

D1

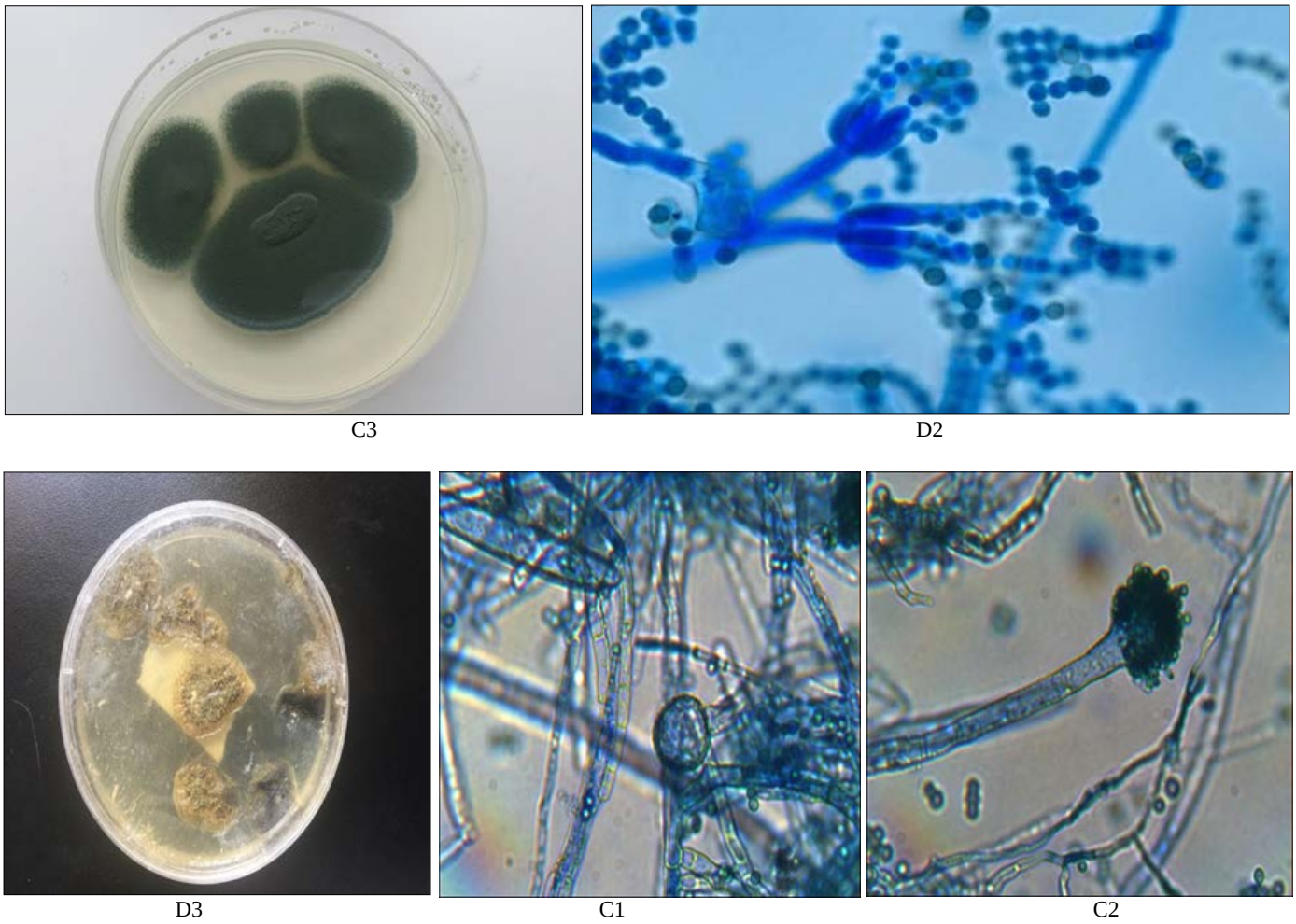

Fig 1: Morphological features of entomopathogens from soil samples and cockroaches

Discussion of Results

Conidial cells are minute cylindrical spores characterized by insulated end which form chains (Figure 1, D1). These morphological features observed was in tandem with the genus *Geotrichum*. The colonies gotten from D2 and C3 isolates were with white edges. This finding also revealed that D2 and C3 have structures that resembles that of a brush. The conidia were cylindrical and chain-shaped located at the end of a single phialide [12].

Morphological characteristics observed for the D2 and C3 isolates was agrees with findings of Thomas *et al.* [11] in which *Penicillium* genus was characterized by its nature hyphae septa, conidia, sterigma as shown in Figure 1. In addition, other features of *Penicillium sp.* observed was having branched mycelium, clustered sterigma, and conidia forming chains [11]. Isolates C3, D1 and D2 were identified by insulated hyphae with a diameter of 8.5 µm. Its conidia are round in nature with the end looking like that of pumpkin-shaped phialide (Table 2). This features tallies with the genus *Aspergillus* sp (Figure 1, C3, D1, D2). Microscopical features of genus *Aspergillus* resembles a bluish-green round-shaped conidia, while the head of the conidia (vesicle) is round-shaped [12].

Based on the noticeable features, colonies of *Aspergillus* sp have distinct margin covered with fluffy well developed aerial plane mycelium on the surface and culture appears yellow green when young and turns jade green as the culture ages. In the same manner, according to other literatures, *Aspergillus sp.* had sterigmata which seemingly cover the upper half part of the vesicles with a serrated surfaces [13-15].

Conclusion

From this research, three fungi isolates were obtained from soil samples which was denoted as D1, D2, D3 and identified as *Geotrichum specie*, *Penicillium specie* and *Aspergillus specie*. Similarly, fungal isolates from *Periplaneta Americana* includes C1, C2, identified as *Aspergillus specie* and C3 belonging to the genus *Penicillium.*

Recommendation

The fungal species isolated should be confirmed through advanced techniques and also should be used as mycoinsecticides in the combat against mosquitoes and various insect pests.

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