Acta Entomology and Zoology

E-ISSN: 2708-0021
P-ISSN: 2708-0013
www.actajournal.com
AEZ 2024; 5(1): 144-149
Received: 22-01-2024
Accepted: 24-02-2024

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Fungi associated with household cockroaches (*Periplanata Americana*: Blattodea: Blattidae): In Akungba environment, Ondo State, Nigeria

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DOI: https://doi.org/10.33545/27080013.2024.v5.i1b.134

Abstract
Cockroaches are implicated in the mechanical transmission of diseases due to their migratory habits from pathogen-dense environments such as sewers to households. This study isolated and characterized fungi in different stages and from various parts of imago of *Periplanata Americana* collected from households in Akungba-Akoko, Ondo. Isolation of different fungi species was carried out by pour plating technique after a five-fold serial dilution using Sabouraud Dextrose Agar (SDA). A total of 22 isolates belonging to 9 fungi genera and 15 species were obtained. *Aspergillus* had the highest frequency of isolation (7; 31.82%). Other fungi genera isolated were *Cryptococcus*, *Rhodotorula*, *Candida*, *Geotrichum*, *Chrysoporium*, *Mucor*, *Penicillium* and *Syncephalastrum*. This study support that cockroaches are implicated in the mechanical transmission of fungi some of which are potential pathogens and may cause diseases. Thus, cockroaches must be controlled and their direct contact with food and kitchenware should be prevented.

Keywords: Public health entomology, vector-borne pathogens, vector ecology, epidemiology, microbiology

Introduction
Among the over 4,600 species of cockroaches worldwide, only about 30 species are syntrophic dwelling within a wide range of habitats and feeding on a vast variety of substances from different sources. The most common among the syntrophic cockroaches are *Periplanata Americana* (L.), (American cockroach), *Blattella germanica* (L.) German cockroach), and *Blattella orientalis* (L.) (Oriental cockroach) (Salehzadeh *et al*., 2007) [39] with majority of them living in the tropics and subtropics and feeding on human food, feces amongst other food sources thereby spreading pathogens such as bacteria, fungi and parasites within human habitations (Isaac *et al*., 2014) [17]. Mechanical transmission of these pathogens potentially occurs through regurgitation, physical dislodgement, and fecal deposition on human food when the cockroaches contact them. (Graczyk *et al*., 2005) [13]. Cockroaches are attracted to moist environments preferring dark and warm conditions such as those found in kitchens, pantries, basements, and toilets (Edwin, 2019) [9]. They can traffic from regions laden with pathogens such as sewers to homes (Rabito *et al*., 2017) [37].
Even though syntrophic roaches are domiciliary, they grow and reproduce in sewers as the environment is suitable for them (Nasirian and Salehzadeh, 2018) [29]. Ignorance, overcrowding, poor housing infrastructure, insufficient dumpsites, and domestic waste accumulation contribute to the yearly increasing infestation of cockroaches in human dwelling (Pan and Zhang, 2019) [34]. Parasite spread by cockroaches pose a challenge to public health especially in developing countries as mechanical transmission of parasitic diseases by cockroaches are often neglected even though they serve as potential vectors or reservoirs of intestinal parasites (Yaro, 2015; Freeman *et al*., 2016; Ngwawe, 2017, Brenner and Kramer, 2019) [40, 11, 31, 4]. Cockroaches can spread fungi, some of which are potential pathogens (Pai *et al*., 2004; Lemos *et al*., 2006) [33, 23], making them ideal vectors of many medically significant fungi such as *Aspergillus* spp., *Candida* spp., *Cryptococcus* spp., *Penicillium* spp among others (Chitsazi *et al*., 2013; Dehghani *et al*., 2014) [6, 8]. These, *Aspergillus* spp. and *Candida* spp. are more prevalent and closely linked with healthcare-associated infections.

(Maschmeyer *et al*., 2007; Bouza and Munoz, 2008) [26, 3]. Madania *et al.* (2023) [25] isolated 62 yeasts and 31 moulds from the surfaces, hemocoel, and digestive tracks of household cockroach. Mloka *et al.* (2022) [27] isolated 7 medically important fungal genera including *Aspergillus*, *Penicillium*, *Candida* and *Mucor* from the external surfaces of cockroaches. This research was carried out to isolate and characterize fungi species on various parts of cockroach imago, as well as in nymph and egg cases laid in different residential areas of Akungba-Akoko, Ondo state, Nigeria.

2. Materials and Methods

2.1 Sample Collection

Cockroach samples (53 eggs, 28 nymphs and 24 imago) were randomly collected from households in Small Gate, Blue House and Permanent Site, Akungba-Akoko, Ondo state, Nigeria. Sterile universal bottles were used to store cockroach eggs picked up aseptically from where they were laid. Nymphs and imago were aseptically collected from kitchens, rooms, and toilet areas of the selected households and put into sterilized containers. The sample were labelled with isolate codes: EG, NYM and IMG and were transported aseptically to the Microbiology laboratory, Adekunle Ajasin University, Akungba-Akoko, Ondo State for analysis. The cockroach samples were identified and authenticated as *Periplanata Americana* in the Department of Animal and Environmental Biology, Adekunle Ajasin University Akukgba-Akoko, Ondo state, Nigeria.

2.2 Media Preparation

Sabouraud Dextrose agar (SDA) was used. Sabouraud Dextrose agar (SDA; 32.5 g) was weighed on aluminium foil with a weighing balance and transferred into a conical flask. 500ml of distilled water was poured and shaken. The media was sterilized using an autoclave and allowed to cool to about 45 °C. Chloramphenicol was aseptically added to inhibit bacteria growth.

2.3 Serial Dilution

Twenty-five (25) test tubes were arranged in a test-tube rack. Each test tube contained 9ml of sterile distilled water. The insect samples were grouped into egg (EG), nymph (NYM), while the imago was dissected using sterilized forceps and blades into mouth and antenna (M&A), limbs and wings (L&W), and abdomen and feces (AB&F). One gram of each sample was weighed using a weighing scale and ground using a sterilized mortar and pestle. One gram of each sample was then aseptically transferred to the sterilized test tubes and thoroughly shaken to ensure even mix. One millilitre of the diluent was transferred aseptically until a 5-fold dilution was obtained. Aseptic transfer of 0.5ml of inoculum of diluent 3 and 5 into sterile Petri dishes preceded pour-plating. Pour-plating was carried out on plates containing the inoculum of the selected diluents with swirling done to ensure even mix of media and inoculum. The media was then left to set. It was then incubated at 25^0C for 3 days.

2.4 Isolation and Sub-culturing of fungi growth

Fungal growth showed different colonies with differing morphological features. Sub-culturing was carried out on freshly prepared SDA to isolate fungal colonies in pure form. Each subculture was then aseptically transferred to slants in McCartney bottles where long time storage occurred. Slants were properly labelled and stored at 4^0C in the refrigerator.

2.5 Characterization of Pure Isolates

Identification of fungi was performed by observing various aspects of colony morphology, characteristic microscopic structures, rate of growth, media which supports the organism's growth, and source of specimen. Yeasts grow as creamy to white colonies. Moulds grow as filamentous colonies of various colours. Macroscopic assessment was done on each isolate according to the method of Kumar *et al*., (2010) [21]. Lactophenol cotton blue (LCB) dye was used to stain fungal spores. The fungal genera were further identified by using various manuals and monographs (Kumar *et al*., 2010) [21].

3. Results

The colony counts of the samples showed that limbs and wings (L&W) of the imago had the highest count (3.7×10^7 SFU/ml), nymph (NYM) had a count of 6.0×10^6 SFU/ml, egg (EG) had a count of 5.0×10^6 SFU/ml while both mouth and antenna (M&A) and Abdomen and Feces (Ab&F) had a count of 2.0×10^6 SFU/ml each.

A total of 22 isolates belonging to 9 fungi genera and 15 species were obtained. The species isolated include: *Aspergillus calidoustus, Aspergillus candidus, Aspergillus flavus, Aspergillus fumigatus, Aspergillus parasiticus, Candida glabrata, Candida parapsilosis, Chrysoporium xerophilum, Cryptococcus laurentii, Cryptococcus neoformans, Geotrichum candidum, Mucor circinelloides, Penicillium digitatum, Rhodotorula minuta,* and *Syncephalastrum racemosum.* (Table 2).

Percentage occurrence showed that *Aspergillus* had the highest frequency of isolation (7; 31.82%), *Cryptococcus* (4; 18.18%); *Rhodotorula* (3; 13.64%); *Candida* and *Geotrichum* (2; 9.09%); while *Chrysoporium, Mucor, Penicillium* and *Syncephalastrum* had the least frequency of isolation (1; 4.55%), (Table 3).

Cryptococcus laurentii, *Aspergillus fumigatus* and *Rhodotorula minuta* had the highest range of distribution (3 in 5 samples), *Geotrichum candidum* (2 in 5 samples), while the others isolates were found in one sample each (Table 4).

Based on number of fungi species isolated, mouth and antenna had the highest (36%), egg (23%), limbs and wings (18%), nymph 14%, while abdomen and feces has the least number of isolated fungi species (9%) (Figure 1).

Table 1: Number of colonies and spore-forming Unit/mL (SFU/mL) of fungi isolates from various parts of cockroaches in akungba

Isolates	Dilution factor	No of Colonies	Spore Forming Unit (sfu/ml)
EG	10^5	5	5.0×10^6
NYM	10^5	6	6.0×10^6
M&A	10^5	2	2.0×10^6
L&W	10^5	37	3.7×10^7
AB&F	10^5	2	2.0×10^6
Control	Blank	No growth	-

*EG (EGG), *NYM (NYMPH), *M&A (Mouth and Antennae), *L&W (Limbs and Wings), *AB&F (Abdomen and Feces)

Table 2: Identification of isolates by microscopy

S/N	Growth rate	Diameter of colony after 7 days on SDA	Pigmentation during Growth	Hyphae form	Conidium form	SPECIES
1	Fastidious	8cm after 5 days	Creamy	Pseudohypha formed	-	*Cryptococcus laurentii*
2	Fastidious	8cm after 5 days	Creamy	Pseudohypha formed	-	*Cryptococcus neoformans*
3	Fastidious	7cm after 5 days	White to Grey	Vegetative hyphae hyaline, branched, septa produced in old cultures	Sporangiophores with strongly curved lateral branches	*Syncephalastrum racemosum*
4	Fastidious	5cm after 3 days	Pale yellow to Brown	Phialides cylindrical with short distinct necks	Conidiophores arise from aerial mycelium	*Aspergillus candidus*
5	Fastidious	3cm after 3 days	Creamy	Phialides with short necks, often greenish pigmented	Conidiophores slightly coloured, short, green and smooth-walled	*Aspergillus fumigatus*
6	Moderate	6cm after 5 days	Creamy	Pseudohypha formed	-	*Rhodotorula minuta*
7	Fastidious	7m after 5 days	White	Advancing hyphae septate, dichotomously branched	Conidia formed by breaking up fertile hyphae and mostly erect	*Geotrichum candidum*
8	Moderate	6cm after 5 days	Cream to brown	Phialides cylindrical tapering to a distinct neck	Conidiophores long, hyaline, smooth and long-walled	*Aspergillus parasiticus*
9	Moderate	6cm after 5 days	Creamy	Pseudohypha formed	-	*Candida glabrata*
10	Moderate	6cm after 5 days	White	Vegetative hyphae rare in mature cells	Clamydiospores borne on hyphal tips or in short side chains of cells	*Chrysoporium xerophilum*
11	Fastidious	8cm after 5 days	Creamy	Phialides cylindrical tapering to a distinct neck	Conidiophores long and coarse	*Aspergillus flavus*
12	Fastidious	8cm after 5 days	Yellow to Brown	Phialides solitary, cylindrical and variable in size	Conidiophores hyaline and irregularly branched	*Penicillium digitatum*
13	Fastidious	8cm after 5 days	Yellow to white	Pseudohypha formed		*Candida parapsilosis*
14	Fastidious	5cm after 4 days	Creamy	Collumella obovoid to ellipsoid in large sporangia	Sporangiophore tall and branched	*Mucor circinelloides*
15	Fastidious	3cm after 3 days	Brown	Phialides cylindrical with a short neck	Conidiophore smooth-walled and hyaline	*Aspergillus calidoustus*

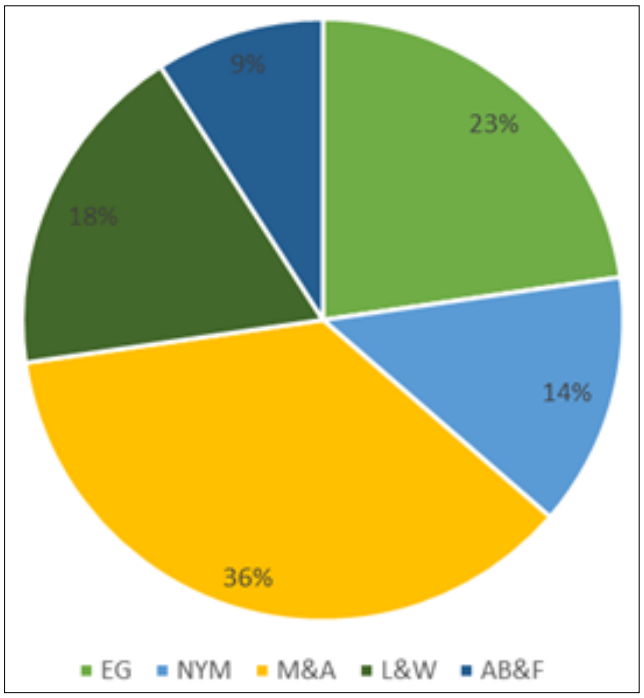
Table 3: Percentage occurrence of fungi isolates (Genera) from various parts of cockroaches in Akungba

Genus	Frequency of Occurrence	Percentage occurrence (%)
Cryptococcus	4	18.18
Syncephalastrum	1	4.55
Aspergillus	7	31.82
Rhodotorula	3	13.64
Geotrichum	2	9.09
Candida	2	9.09
Chrysoporium	1	4.55
Penicillium	1	4.55
Mucor	1	4.55
	Total = 22	Total = 100

Table 4: Occurrence of fungi isolates at various stages and in different parts of the cockroach

Isolates	EG	NYM	M&A	L&W	AB&F
Cryptococcus neoformans	+	-	-	-	-
Cryptococcus laurentii	+	+	+	-	-
Syncephalastrum racemosum	+	-	-	-	-
Aspergillus candidus	+	-	-	-	-
Aspergillus fumigatus	+	-	+	-	+
Aspergillus parasitucus	-	-	+	-	-
Aspergillus flavus	-	-	+	-	-
Aspergillus calidoustus	-	-	-	+	-
Rhodotorula minuta	-	+	+	+	-
Geotrichum candidum	-	+	+	-	-
Candida glabrata	-	-	+	-	-
Candida parapsilosis	-	-	-	+	-
Chrysoporium xerophilum	-	-	+	-	-
Penicillium digitatum	-	-	-	+	-
Mucor circinelloides	-	-	-	-	+

*EG (EGG), *NYM (NYMPH), *M&A (Mouth and Antennae), *L&W (Limbs and Wings), *AB&F (Abdomen and Feces)



*EG (EGG), *NYM (NYMPH), *M&A (Mouth and Antennae), *L&W (Limbs and Wings), *AB&F (Abdomen and Feces)

Fig 1: Pie chart of percentage isolation of fungi isolates in each sample

4. Discussion

Periplaneta Americana can carry fungi of public health importance and therefore can cause health hazards as a mechanical vector (Fakoorziba *et al*., 2010; Hashemi-Aghdam and Oshaghi, 2015) [10, 14]. The presence of cockroaches in sensitive environments (such as houses or hospitals) are more dangerous than their presence in other areas and can affect environmental, people and community health. Population density and feeding habits make cockroaches an implicated vector of pathogenic micro-organisms.

The study showed that cockroaches in residential areas were contaminated with fungi of medical importance. The main fungi isolated were species of *Aspergillus* (31.82%), *Cryptococcus* (18.27%) and *Rhodotorula*. In a study by Kassiri *et al*., (2018), the main fungi isolated were species of *Candida* spp. (41.4%), *Aspergillus* spp. (37.1%), *Rhodotrula* spp. (27.1%) and *Penicillium* spp. (11.4%). In another study by Lemos *et al*. (2006) [23], *Candida* spp (38.6%), *Aspergillus* spp (30.7%) and *Penicillium* spp (8.9%) were the most frequently isolated fungi genera. (Hernández-Rosas *et al*., 2019) [16]. The percentage occurrence of fungal species in different studies may be associated with the range of physical environments in which the collected samples migrated from. In this study, 59% of the isolates were filamentous and 41% were yeast while in a study by Kissiri *et al*. (2018) [20] 52% of the isolates were filamentous and 48% were yeast and 88.68% of cockroaches carried fungi of economic importance including *Penicillium* spp, *Candida* spp, and *Aspergillus* spp. The source of food of cockroach sample can affect the composition and diversity of microorganisms isolated on their external surfaces.

Aspergillus had the highest frequency of isolation (31.82%) in this study. *Aspergillus calidoustus, A. candidus, A. flavus, A. fumigatus and A. parasiticus* were the five species of *Aspergillus* identified in this study. *Aspergillus Niger* and *A. flavus* have been reported from patients with invasive disease (Person, 2010; Park *et al*., 2012) [36, 35]. Aspergillosis is common in bone marrow transplant recipients and patients with lung disorders. In Immunocompromised patients, obstructive bronchial aspergillosis, allergic Aspergillus tracheobronchitis, and pulmonary aspergilloma are reported (Li *et al.*, 2008; Nabili *et al*., 2013) [24, 28]. *A. flavus* has been isolated in bone marrow transplant patients (De La Rosa *et al*, 2002) [7].

Candida glabrata and *Candida parapsilosis* were the two *Candida* species identified in this study. The study by Lemos *et al*. (2006) [23] in Brazil isolated *Candida glabrata* (42.2%) and *C. magnoliae* (17.8%). Another study by Saichua *et al*. (2008) [38], isolated *Candida glabrata, C. parapsilosis, C. pseudotropicalis* and *C. albicans*. Kissiri *et al*. (2018) [20] identified *Candida albicans, C. glabrata* and *C. famata*. *Candida* is an opportunistic pathogenic agent, due to its ability to colonize the human body (mouth, skin, genitourinary tract and gut). It causes candidiasis and other symptoms when a weakened immune system or other factors allow it to grow unabated (Cheng *et al*., 2012) [5].

Cryptococcus laurentii and *Cryptococcus neoformans* were isolated from this study. *Cryptococcus neoformans* and *C. gattii* commonly cause diseases known as Cryptococosis in humans. *Rhodotorula minuta* and *Syncephalastrum racemosum* causes opportunistic infections in Immunocompromised patients (Henrich, 2009) [15] while *Geotrichum candidum* is a commensal of the skin, trancheobronchial tree and gastrointestinal tract (Bonifaz *et al*., 2010) and a rare human pathogen with low virulence causing local disseminated disease called geotrichosis (Henrich, 2009) [15].

Chrysoporium spp generally have weak pathogenic ability. The members of the genus *Chrysoporium* are common soil saprobes that occasionally cause systemic infections. Gopal *et al*. (2020) [12] reported a case of pulmonary disease due to *Chrysoporium* spp in a pre-existing tuberculosis cavity in an Immunocompromised male patient. The fungus was isolated from the broncheoalveolar lavage fluid and the patient recovered with an anti-tuberculosis treatment.

One *Penicillium* species was isolated from this study: *Penicillium digitatum*. *Penicillium* spp are known pathogens of fruits (citrus) and are usually considered non-pathogenic to humans. However, in Immunocompromised hosts they can be virulent pathogens and cause death. (Oshikata *et al.*, 2013) [32]. *P. digitatum* very rarely causes systemic mycosis in humans. Oshikata *et al*. (2013) [32] reported a case of fatal pneumonia due to *P. digitatum* infection which was confirmed by repeated examination of cultured sputum.

Mucor circinelloides is one of the causative agents of mucormycosis which is an uncommon but frequently lethal fungal infection of humans (Lee *et al*., 2013) [22]. Mucormcycosis is an emerging fungal infection often affecting Immunocompromised and vulnerable populations including patients with diabetes, AIDS, hematologic malignancies or trauma.

High microbial load in/on household cockroaches can transmit fungal infections particularly in homes and hospitals. The presence of cockroach in sensitive areas is a threat to public health and their control must be prioritized to keep these environments safe (Kissiri *et al*., 2018) [20]. There is need to prevent contact of cockroaches particularly with food, fruits, and all kitchenware particularly those with

food scraps as well as clean utensils kept in plate racks, cupboards, or open storage buckets.

5. Conclusion

The study showed that pathogenic filamentous fungi and yeast can be isolated from the external surfaces of cockroach eggs, nymphs and various parts of imago (mouth, antennae, limbs, wings, abdomen and feces). Cockroaches thus serve as vectors of fungi in residential environments.

Many studies on the role of cockroaches in the mechanical transmission of diseases focuses mainly on the imago. There is need for further research on how nymphs can potentially transmit pathogens and the roles of food contamination by cockroach eggs usually laid in many places including cupboards, vertices of walls, plate racks amongst others. Research on cockroach control should focus more on driving cockroaches farther away from residential areas as these promises to be a much more effective strategy than killing cockroaches. Environments in all residential area must always be kept clean as proper sanitation reduces the need for excessive insecticide usage which is not environmentally friendly and can result in insecticide resistance.

6. Acknowledgments

We would like to thank the Department of Animal and Environmental Biology, Adekunle Ajasin University, Akungba-Akoko, Ondo State, for identifying the cockroach species used in this research work.

7. Funding

This work was not supported by any grants or funding.

8. Conflict of interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

9. Authors Contribution

ES and FA conceived the idea. EO devised the methodology and interpreted results. ES carried out the experiment under supervision by FA and EO All authors contributed to the final manuscript.

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