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Isolation and characterization of pathogenic fungi from the body surface of adult cockroach *Periplaneta americana* in Kirkuk governorate, Iraq

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Abstract
Cockroaches, particularly the American cockroach (*Periplaneta americana*), are known as carriers of various microorganisms, including fungi, due to their preference for warm and humid environments rich in organic matter. This study focuses on the isolation and characterization of fungi associated with adult *P. americana*, L. (Orthroptera: Blattidae), shedding light on potential fungal pathogens or symbionts that could influence the health and possibly impact human environments, which was taken from Kirkuk governorate homes, swages, and menhols. 60 insects were collected from four areas: Alhawja City, Arafa, Shoraw, and Hai-Alnasr. The insects were taken with a hole body, superficially sterilized, and incubated on SDA nutrient medium. 26 isolates were collected, and eight species of fungi were isolated. The identified fungal isolates include *Alternaria alternate*, *Aspergillus niger*, *A. flavus*, *Beauvaria spp*., *Candida spp*., *Penicillum sp*., *Aspergillus fumigatus, and Rhizopus spp*. It was carried on parts of the insect's body; the study also showed that *A. niger* was the most common fungus isolated with a density of 19.23% and *Candida spp*. With the same density.

Keywords: *Periplaneta americana*, aflatoxins, *Aspergillus niger*, *Aspergillus fumigatus*

Introduction
Most microorganisms, which have been excluded from cockroaches, are transmitted to humans via the consumption of contaminated water and food [1]. Examining medically significant insects, including cockroaches and flies, that pose a threat to human health by acting as carriers of fatal illnesses like malaria, leishmaniasis, dengue fever, and diarrhea is known as medical entomology [2]. Also infect plants and animals, like the fungus Fusarium oxysporium that infects agricultural plants [3]. Because of their filthy habits and habitats [4, 5], cockroaches are implicated in the mechanical dissemination of a wide range of food-borne infections [6], contributing to the rise in chronic diseases in the communities of underdeveloped nations [7]. In order to isolate and measure the amount of fungi present in the American cockroach *(Periplaneta americana*), the study's design involved collecting the insects and utilizing microscopical and biochemical techniques. Since the house environments provide them with suitable temperature, humidity, and a ready source of food, the presence of cockroaches there is not uncommon [8]. Understanding the fungal communities harbored by cockroaches is crucial for assessing potential risks to human health [9]. Cockroaches can act as mechanical vectors for pathogenic fungi, potentially spreading them in residential and commercial settings. Moreover, the presence of fungi capable of infecting cockroaches themselves may influence the insects' population dynamics and behavior, affecting their role as pests. The present study was conducted to isolate and identify fungi from the external surfaces of the cockroaches (*Periplaneta americana*). Although previously we discussed some of the molds and yeasts associated with American cockroaches using morphological characteristics that were collected from different areas of Kirkuk governarate.

Isolation Methodology
This study was conducted in the college of education for women's labrotories of Kirkuk University. From the period August to November 2023. The test group of insects was captured (mostly at night or in the early morning) from urban environments.

60 insects were collected and checked under the anatomy chamber. The American cockroach infestation was diagnosed together on a classification key related to insects. [8] Cockroaches were also collected from kitchens, basements, or bathrooms of residential areas, and their microbial flora was studied. Samples were taken to the laboratory and kept in the fridge to immobilize insects and dissected under sterile conditions. Each cockroach was collected in sterile test tubes (Fig. 1). And fungal isolates were obtained from surface body parts, including the exoskeleton, gut, and respiratory openings (spiracles). Insect parts were sterilized by sodium hypoclorite (NaOCl) with a 1% concentration for 3 minutes to eliminate the microbes from the surface and to slow the fungi from growing into the insects body parts. After that, insects transferred to tubes containing sterilized distle water to remove sodium hypoclorite, and then insects were inculcated on petri dishes. With medium (SDA) on 25 c for 5 days, then the petri dishes tested to see the fungal colonies and frequency of fungi tested by:

Density of fungi (%): (number of fungal isolates ÷ total number of all fungi × 100) (1)

The fungal isolate was identified using the cultural characteristics of the colonies that grew on (SDA). Figure (2) show the growth. A portion of the fungal growth was then loaded onto slides and stained with lactophenol cotton blue stain to study the morphology characteristics such as spores and gonidiophores. The slides were then examined under a microscope and identified using a classification key.

Fig 1: Insects in test tubes

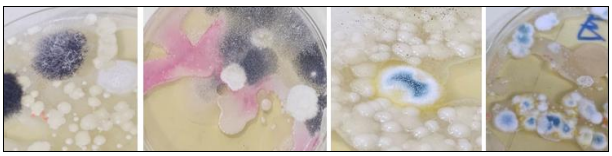

Fig 2: The first culture results

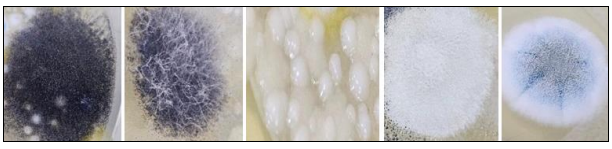

Fig 3: Types of fungi after purification

Detection of the ability of fungi that were isolated from the external body of *Periplaneta americana* to produce aflatoxines

Aflatoxin is a very poisonous secondary metabolite that is mostly produced by several Aspergillus fungi, the most important of which being *Aspergillus flavus* and *Aspergillus parasiticus*. Understanding the secretion processes, effects, and management techniques of these mycotoxins is critical since they pose substantial health concerns to both humans and animals [12]. Method used in 1999 satio and machida. This is accomplished by putting the growing media in dishes. After autoclaving to sterilize, inoculate potato dextrose agar. Transferring a portion of the fungal colonies from the dishes using a sterile needle, each colony was moved to the medium on each plate, and the inoculation plates were then incubated. Two and a half days: the infected plates were kept at 25 °C in the incubator for four to seven days. Following the infected plates' incubation Two and a half days: the vaccinated plates were incubated at 25 °C for four to seven days. The plates were removed from the incubator and given a little shake once the incubation period had ended. Each plate then received 0.2% of a 25% concentration of ammonia solution. Then, for a duration of two to seven days, all the plates were put back into the incubator. The colony colors were monitored every day while the incubator was kept at a temperature of 25 °C. The colony's base changing to a reddish-pink or yellow-orange hue with varying tones is indicative of secretion.

Fungi ability to produce hemolysis as a virulence factor

The likelihood that fungi evolved hemolysis for the sole purpose of lysing red blood cells *in vivo* to improve growth is highly unlikely. Most fungi exist in the environment as saprophytes. In some cases, the fungus may grow on or in living tissues, especially in an immune-suppressed individual. This may provide an opportunity for colonization and infection. For fungi, animal hosts are a rich source of organic material. As noted above, recent studies of *A. fumigates* cause one to question whether fungal hemolysis has any demonstrable role in pathogenesis [13]. We use in this study blood agar medium to identify the fungus that produces hemolysis by taking a small amount of fungal colonies and culture them with cork penetrator at 59 °C for 14 days. The results are performed on the basis of the distance of the lyse area and the time recorded [14].

Results and Discussion

American cockroaches have been shown to harbor over 100 different species of bacteria, fungus, and parasites, making them potential carriers of these pathogens [16]. Cockroaches may spread infection through the fecal-oral pathway because they feed on dirt and excrement [15]. They are unquestionably carriers of organisms that cause food poisoning, typhoid, diarrhea, and dysentery, yet they are occasionally not treated very seriously [17]. The results of the present study revealed contamination of almost all 60 cockroaches with 15 insects collected from 4 different areas of Kirkuk ggovernorate, which are significantly higher in comparison to those collected from other areas. Table (1) show the areas, the number isolates, tes and the types of fun I The he most common fung were as found *in A. ngier* and *Candida spp*. Followed by *A. fumigatus* and *Rhizopus spp*., *Penicillium spp*., when the other fungi that occur achieved low density like *Rhizopus spp*., *Penicillium spp*., and

Beauvaria spp. We can use this fungus to biocontrol some insects; increasing reliance on synthetic pesticides necessitates the exploration of eco-friendly alternatives. Because of the extreme congestion in that location and the lack of seriousness with which these insects are dealt with, cockroaches are more prevalent in one area than in another. These insects can cause contamination with a variety of germs. The ability of these insects to develop a high resistance to insecticides and methods of control, coupled with their preference for humid, moderately warm environments, as well as their locations in bathrooms, kitchens, and warehouse crevices, along with the lack of cleaning of abandoned places, all point to the necessity of using environmentally friendly biological control. It works well against these insects. These studies confirm that cockroaches carry a lot of fungi on the body surface, like study [18] in Iran hospitals researchers isolate *Penicellium sp*. *Mucor*, *Rhizopus*, *A. niger*, *and A.* fumigatus in India [19]. The genus *Aspergillus* constituted the most commonly isolated entomopathogenic fungi, followed by isolates of *Beauveria*, *Clonostachys*, *Talaromyces*, *Trichoderma*, *Fusarium*, *Aspergillus*, *Candida*, and *Meyerozyma* genera. Also in study [20], researchers isolated fungi from cockroaches, which were taken from the Insect Museum of the Plant Protection Department, Faculty of Agriculture, Omar Al-Mukhtar University, Libya. The identified fungal isolates include *Alternaria altemata*, *Aspergillus niger*, *A. terreus*, *Beauvaria* sp., *Madurella* sp., *Penicillium verrucosum*, *P. commune, and Penicillum* sp.

Table 1: Fungal species isolated from adult cockroach in different areas

S. No.	Fungi species	Hai alnasr	Shoraw	Alhawija city	Arafa	Total
1.	*Penicillium spp.*	1	0	1	2	4
2.	*Rhizopus spp.*	1	1	1	1	4
3.	*Beauvaria spp.*	2	0	0	0	2
4.	*Aspergillus niger.*	1	1	2	1	5
5.	*Aspergillus flavus*	0	0	1	0	1
6.	*Aspergillus fumigatus*	1	2	1	0	4
7.	*Alternaria alternate*	0	0	1	0	1
8.	*Candida spp.*	1	1	2	1	5
	Total	7	5	8	5	26

Results in Table (2) show the fungal density according to their occurance in the samples *A. niger* and *Candida* spp. Have the equal density with 19.23%), followed by *Penicillium spp*. and *Rhizpous spp*. *Aspergillus fumigatus* (15.384%), and the lowest density was *Beuvaria spp*. (7.69%) and *Aspergillus flavus*, *Alternaria alternate* with density (3.84%). These results refer to the commonality of the genera *Aspergillus spp*. *and Candida spp*. A fungus of the genus *Aspergillus* is widespread in the environment. The fungus of *Aspergillus* species has different abilities to produce virulence factors, and the most dangerous species is *A. fumigatus*. Certain *Aspergillus* species most frequently, *Aspergillus fumigatus*, can cause a range of allergy reactions as well as potentially fatal systemic infections in people [22, 23]. The reason behind the emergence of these fungal species on the exterior of the insect's body, even after it was sanitized, could be that internal germs were present in its tissues, including its digestive canal, legs, wings, and mouth parts. This could clarify what [24] said, which is that fungi can pierce any substance so deeply that it is not removed by any disinfectant. Because these fungi can withstand prolonged heat without becoming killed, they are readily available. In order to adapt to the complicated and changeable environment, the gut microbiome may change with the growth and development of *Periplaneta americana*. A good spot to hide during the day is a manhole; in the evening, they all go to different areas of the bathrooms and wastes where they could be exposed to infections on their bodies.

Table 2: Fungal isolates and their Density

Fungi name	N. of Isolates	Density (%)
Penicillium spp.	4	15.384
Rhizpous spp.	4	15.384
Beuvaria spp.	2	7.69
Aspergillus niger	5	19.23
Aspergillus flavus	1	3.84
Aspergillus fumigatus	4	15.384
Alternaria alternate	1	3.84
Candida spp.	5	19.23

Results in Table (3) show the ability of fungal isolates to produce aflatoxins and blood lysis, and they refer to the high ability of *Aspergillus spp*. Mycotoxin production was found to be highly proficient in *Aspergillus niger*, *Aspergillus flavus*, and *Aspergellus fumigutus*, but not in *Rhizopus spp*. *Alternaria* exhibited a middling level of toxicity production, and *Penicellium spp*. and *Candida spp*. showed no toxicity secretion ability at all. Each of the isolates demonstrated the ability to dissolve blood in terms of this ability, with the exception of *Penicellium spp*. and *Candida spp*. The fungal species *Aspergillus niger* was the most hematolytic isolate. With a decomposition diameter of 27 mm and a duration of 3 days, *Aspergillus fumigatus*, a fungus, placed in the second category, while its decomposition time was 5 days and its diameter was 25 mm. With a diameter of 23 mm and a duration of 7 days, the fungus *Aspergillus flavus* ranked third, while *Alternaria alternate* had the least amount of disintegration, measuring only 5 mm and requiring a period

of 7 days. Research shows that important virulence factors that allow fungal isolates of different genera and types to cause infection are present in these isolates. This suggests that the spread of these insects in homes and between waste, kitchens, and sewers contributes to the spread of these medically significant species and the fungi and toxins they carry.

Table 3: Ability of fungal isolates to produce Aflatoxines and hemolysis

Fungi types	Aflatoxin production	Ability of blood lyses	
		Distance(mm)	Time (days)
Penicillium sp	_	0	0
Rhizpous spp.	+	12	8
Beuvaria spp.	_	0	0
Aspergillus niger	++	27	3
Aspergillus flavus	++	23	7
Aspergillus fumigatus	++	25	5
Alternaria alternate	+	5	7
Candida spp.	_	0	0
++ High ability	+Moderate ability	- Non able	

Conclusion

This work emphasizes the significance of researching *P. americana* associated fungal communities in order to comprehend their ecology, their functions as pathogens, and public health implications. 8 species of fungi were isolated and we study some virulence factors. In order to create effective pest management plans and identify any possible health problems related to cockroach infestations in metropolitan areas, more research into the interactions between these fungus, cockroaches, and their surroundings is important.

Conflict of Interest

Not available.

Financial Support

Not available.

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