Erciyes Med J 2017; 39(1): 1-6 • DOI: 10.5152/etd.2017.16067

Culex (Culex) Pipiens Mosquitoes Carry and Harbor Pathogenic Fungi during Their Developmental Stages

ORIGINAL INVESTIGATION

Fatma H. Galal[1,2], Amany AbuElnasr[3], Ibtesam Abdallah[3], Omaima Zaki[3], AlaaEddeen M. Seufi[1,2]

ABSTRACT

Objective: Fungi are the main source of aflatoxin contamination in nature. The present study aimed to assess the role of the cosmopolitan mosquito species, *Culex pipiens*, in the circulation and dissemination of pathogenic fungi in nature, and to evaluate its capability to harbor these fungi.

Materials and Methods: Fungi were isolated and identified from both, the external surface and the internal organs of the developmental stages and from the breeding environment of *Cx. pipiens*.

Results: A total of 35 fungal isolates were isolated from both, the internal organs and the external surface of the developmental stages and from the breeding environment of *Cx. pipiens*. These isolates were identified as eleven *Penicillium notatum* isolates, eleven *Aspergillus flavus* isolates, six *Rhizopus stolonifer* isolates, four *Candida albicans* isolates, two *Fusarium solani* isolates, and one *Aspergillus niger* isolate. Antagonistic activity showed that the *P. notatum* growth inhibited the growth of the bacteria, *Staphylococcus aureus*.

Conclusions: This study revealed that the different developmental stages of *Cx. pipiens* mosquito were capable of harboring many fungal species. Subsequently, this mosquito will be incriminated in the mechanical dissemination, circulation, and transmission of these fungi in nature, during its life cycle.

Keywords: *Cx. pipiens*, mosquitoes, pathogenic fungi, developmental stages, breeding environment

INTRODUCTION

Mosquitoes are unquestionably the most important arthropod vectors of diseases. The maintenance and transmission of pathogens that cause malaria, lymphatic filariasis, and numerous viral infections are absolutely dependent on the availability of competent mosquito vectors (1, 2).

Among the culicine mosquitoes in Egypt, *Culex pipiens* stands first and foremost as a widely distributed species that is frequently encountered in prodigious numbers in many regions of the Egyptian territory. It has also been incriminated in the transmission of the nocturnally periodic bancroftian filariasis in the Nile Delta of Egypt and the Rift Valley fever (1, 2).

Fungi can occasionally attack insects or develop symbiotic relationships (3). Many interactions between fungi and mosquitoes have been reported worldwide (4-10). Other interactions between bees and fungi have been documented, as well (11-13). Termite-fungi relationships have also been observed (14). Other insect-fungi interactions have also been recorded, including lepidopteran-fungi (15), beetle-fungi (5, 16), and triatomine-fungi interactions (17-20).

The main objectives of this study were to assess the role of the cosmopolitan mosquito species, *Cx. pipiens*, in the circulation and dissemination of pathogenic fungi in nature, and to evaluate its capability to harbor these fungi. Antagonistic activity between the isolated fungi and associated bacteria was also investigated.

MATERIALS and METHODS

Colonization of *Cx. pipiens*

A colony of the mosquito, *Cx. pipiens*, was maintained under controlled laboratory conditions (27±2°C, 60-70% Relative humidity (RH) and 10/14 light/dark photoperiod cycle) in the insectary of the Department of Entomology, Faculty of Science, Cairo University, Giza, Egypt, up till now. This colony was used for supplying the immature stages (eggs, larvae, and pupae) of *Cx. pipiens* during this study. Briefly, the eggs were set up to hatch, and the 1st instar larvae were seeded into plastic cups (25´35´7 cm^3) containing water at a constant density of 300 individuals per cup. The

[1]Department of Biology, Aljouf University College of Science, Sakaka, KSA

[2]Department of Entomology, Cairo University Faculty of Science, Giza, Egypt

[3]Department of Botany and Microbiology, Helwan University Faculty of Science, Egypt

Submitted
15.06.2016

Accepted
28.12.2016

Correspondence
AlaaEddeen M. Seufi, Department of Biology, Aljouf University College of Science, Sakaka, KSA; Department of Entomology, Cairo University Faculty of Science, Giza, Egypt
Phone: 00966560067598
e.mail: alaaseufi@yahoo.com

larvae were provided with activated yeast or tetramin, every 2 days, until pupation. Water was changed on feeding days to avoid bacterial growth on the water surface. On pupation, cups were placed inside an emergence cage (27x40x35 cm^3) and provided with a source of 10% sugar solution for the emerged adults (21). All necessary permits for this study were obtained from the local ethics committee of Cairo University. This study did not involve endangered or protected species. The informed consent rules are not applicable for this study.

External fungal isolation

Twenty individuals from *Cx. pipiens* egg rafts, 4th larval instars, pupae, and adults (males and females) were caught using sterile test tubes and sterile hand gloves. Then, they were anesthetized by freezing at 0°C for 5 min. Two milliliters of sterile normal saline (0.9%) was then added to each *Cx. pipiens* sample test tube, and the samples were thoroughly shaken for 2 min. A fixed volume (0.1 mL) of the washing saline was inoculated onto Czapek-Dox's agar medium using a sterilized scalpel, inside the laminar air flow hood. The plates were incubated at 28°C for 5-7 days (22).

Internal fungal isolation

Twenty individuals from *Cx. pipiens* egg rafts, 4th larval instars, pupae, and adults (males and females) were anesthetized using cold 70% ethanol, and then, the insect samples were transferred to centrifugal filters and washed five times with 70% ethanol in order to sterilize the insect surface. The surface-sterilized insects were spun for 5-10 seconds in a table-top microcentrifuge at a low speed setting (around 1,000 *g*) to dispose most of the ethanol. Samples were washed thrice with sterile distilled water. The surface-sterilized samples were homogenized in sterile Ringer's solution using a glass tissue grinder. A suspension of 0.1 ml was spread on Czapek-Dox's agar media using a sterilized scalpel. All steps in the isolation procedure were carried out in the laminar air flow hood to avoid contamination. Plates were incubated at 28°C for 5-7 days (23).

Fungal isolation from the insect environment

A sample of 0.1 mL was collected from the breeding water (immature breeding environment) and sucrose water, and from air (mature breeding environment), and spread separately on Czapek-Dox's agar plates using a sterilized scalpel, inside the laminar air flow hood. For fungal isolation from the air, the plates were exposed to air inside the cage for 30 minutes, incubated at 28°C for 5-7 days, and then, investigated for fungal identification (24).

Characterization of fungal isolates

The isolated fungal colonies were identified according to their macroscopic morphology, and the selected colonies were mounted and stained with lactophenol for light microscopic examination. This was presented according to the method of Arx (25).

Antagonistic activity between isolated fungi and other associated bacteria

Dox medium was prepared and poured into plates. One milliliter of each isolated fungal suspension was inoculated onto the DOX media. On cooling, a disk of filter paper immersed in bacterial suspension was placed on the DOX plate. The tested bacterial strains were previously isolated from the different developmental stages of the mosquito, *Cx. Pipiens* (21). Measurements of growth inhibition zones were recorded and compared to a reference chart after incubation at 30°C for 5-7 days. Each experiment was repeated thrice.

RESULTS

Fungal isolates

A total of 35 fungal isolates were identified during this study. These isolates were isolated from the different developmental stages of the mosquito and from its breeding environment.

External fungal isolation from the insect

Out of the 35 isolates, 11 fungal isolates (31%) were isolated from the external surface of the eggs, 4th larval instar, pupae, the adult male, and the adult female of *Cx. pipiens*. Out of these 11 isolates, 2 (18%), 3 (27%), 1 (9%), 3 (27%), and 2 (18%) isolates were isolated from the egg, larval, pupal, adult male, and adult female stages, respectively.

Results of the fungal identification revealed that we had isolated four different fungal strains (Table 1). Among these 4 fungal strains, *Aspergillus flavus* and *Penicillium notatum* were the most persistent fungi and appeared in four mosquito developmental stages, followed by *Rhizopus stolonifer*, which appeared in two developmental stages (larva and adult male), and *Candida albicans*, which was isolated from the egg rafts and was lost in the other stages. It was observed that *A. flavus, P. notatum*, and *R. stolonifer* were isolated from the larvae and the adult male mosquitoes (Table 1).

Internal fungal isolation from the insect

Out of the 35 isolates, 13 fungal isolates (37%) were isolated from the internal organs of the different developmental stages of *Cx. pipiens*. Out of these 13 isolates, 1 (7.5%), 3 (23%), 1 (7.5%), 4 (30.5%), and 4 (30.5%) isolates were isolated from the egg, larval, pupal, adult male, and adult female stages, respectively.

Results of the fungal identification revealed that no additional fungal strains were identified (Table 2). Among the four fungal strains, *A. flavus* and *P. notatum* were the most persistent fungi and ap-

Table 1. External fungal isolates from eggs, larvae, pupae, and the adult male and adult female

Stages	Eggs	Larvae	Pupae	Male	Female
Fungi					
Aspergillus flavus	√	√		√	√
Candida albicans	√				
Penicillium notatum		√	√	√	√
Rhizopus stolonifer		√		√	

√: appearance

Table 2. Internal fungal isolates from eggs, larvae, pupae, and adults (males & females)

Stages	Eggs	Larvae	Pupae	Male	Female
Fungi					
Aspergillus flavus	√	√		√	√
Candida albicans				√	√
Penicillium notatum		√	√	√	√
Rhizopus stolonifer		√		√	√

√: appearance

peared in four mosquito developmental stages, followed by *R. stolonifer*, which appeared in three developmental stages (larva, adult male, and adult female), and *C. albicans*, which was isolated from the adult stages (male and female) and lost in the immature stages. The four fungal strains, *A. flavus*, *C. albicans*, *P. notatum*, and *R. stolonifer*, were isolated from both, the male and female adult mosquitoes (Table 2).

Isolation from the mosquito environment

Out of the 35 isolates, 11 fungal isolates (31%) were isolated from the environmental sources of the immature and mature stages of *Cx. pipiens*. Out of these 11 isolates, 3 (27%), 3 (27%), and 5 (45%) isolates were isolated from air, sucrose water, and breeding water, respectively.

Table 3. Fungal isolates from environmental sources (air, sucrose water, and breeding water)

Environmental sources	Fungi
From air	*Penicillium notatum*
	Aspergillus flavus
	Fusarium solani
From sucrose water	*Aspergillus flavus*
	Penicillium notatum
	Rhizopus stolonifer
From breeding water	*Aspergillus flavus*
	Penicillium notatum
	Aspergillus niger
	Candida albicans
	Fusarium solani

Results of the fungal identification revealed that one additional fungal strain (*Fusarium solani*) was identified from the air and breeding water samples (Table 3). Among the five identified fungal strains, *A. flavus* and *P. notatum* were the most persistent fungi and appeared in all the breeding environments (air, sucrose, and water), followed by *F. solani*, which appeared in the air and water environment. *C. albicans* and *Aspergillus niger* were isolated from only the breeding water environment, whereas *R. stolonifer* was isolated from only the sucrose water environment (Table 3).

Common fungal isolates between external and internal isolates

It was observed that all the fungal strains shared the external and internal isolations, except *C. albicans*. It did not share the internal isolation in the egg stage and the external isolation in the adult stages (male and female) with the other fungal strains. In addition, *R. stolonifer* did not share the external isolation in the adult female stage (Table 4). It was clear that *A. flavus* was the most prevalent strain that appeared in all isolations (external and internal). However, *A. flavus* was not isolated from the pupal stage at all (Table 4). *P. notatum* was not isolated from the egg stage. Meanwhile, *P. notatum* was the most prevalent strain that appeared in all other stages (larva, pupa, male, and female) in both, external and internal isolations (Table 4). *R. stolonifer* was the third strain that shared external and internal isolations in the larval and adult male stages (Table 4).

Characterization of Fungal Isolates

Macroscopic characterization

A total of 35 fungal isolates were identified from both, the internal organs and the external surface of the different developmental stages, and from the breeding environment of the mosquito, *Cx. pipiens*. The fungal isolates were identified macroscopically as six

Table 4. The common fungal isolates between external and internal isolates

Stage	External	Internal	Common
Eggs	*Aspergillus flavus*	*Aspergillus flavus*	*Aspergillus flavus*
	Candida albicans		
Larvae	*Aspergillus flavus*	*Aspergillus flavus*	*Aspergillus flavus*
	Penicillium notatum	*Penicillium notatum*	*Penicillium notatum*
	Rhizopus stolonifer	*Rhizopus stolonifer*	*Rhizopus stolonifer*
Pupae	*Penicillium notatum*	*Penicillium notatum*	*Penicillium notatum*
Male	*Aspergillus flavus*	*Aspergillus flavus*	*Aspergillus flavus*
	Penicillium notatum	*Penicillium notatum*	*Penicillium notatum*
	Rhizopus stolonifer	*Rhizopus stolonifer*	*Rhizopus stolonifer*
		Candida albicans	
Female	*Aspergillus flavus*	*Aspergillus flavus*	*Aspergillus flavus*
	Penicillium notatum	*Penicillium notatum*	*Penicillium notatum*
		Rhizopus stolonifer	
		Candida albicans	

Table 5. Microscopic and macroscopic characterization of fungal isolates

Fungi	*Penicillium notatum*	*Fusarium solani*	*Aspergillus niger*	*Aspergillus flavus*	*Rhizopus stolonifer*	*Candida albicans*
Branching	Highly branched	Short multi-branched	Dichotomous branching	Thread-like branching	Branched	Non-branching Globular structures
Hyphae	Septate colorless hyphae	Septate	Septate and hyaline	Septate, hyaline, colorless, and rough	Non-septate Special strands of hyphae connecting fungal bodies can be called stolons	Pseudohyphae
Conidiophores	Branched	Fusiform, cylindrical, often moderately curved with an indistinct pedicellate foot cell and a short, blunt apical cell	Conidiophores were long, smooth, and hyaline, becoming darker at the apex	Coarsely roughened, uncolored, with vesicles, spherical, and metulae covering nearly the entire vesicle	Sporangiophores appear	
Spores	Conidiospores are the main dispersal route for the fungi, and often are green in color	Hyaline, globose, smooth to rough-walled, borne singly or in pairs	Numerous black	Yellow-green spores	Sporangia are globose, often with a flattened base, grayish black, powdery in appearance, and with many spores	Reproduction by budding
On agar plate	Granular, flat, often with radial grooves, yellow at first, but quickly becoming bright to dark yellow-green with age	White to cream, becoming bluish-brown when sporodochia are present	Structures with numerous black dots	Yellow-green colony	Fill the culture plate with dense, cottony, aerial mycelium, at first white, then becoming gray	White colony

different strains: *P. notatum* (11 isolates), *A. flavus* (11 isolates), *R. stolonifer* (6 isolates), *C. albicans* (4 isolates), *A. niger* (one isolate), and *F. solani* (2 isolates).

On an agar plate, *P. notatum* appeared granular, flat, often with radial grooves, yellow at first, but quickly becoming bright to dark yellow-green with age. *A. flavus* was observed as powdery masses of yellow-green spores on the upper surface and reddish-gold on the lower surface. *R. stolonifer* was observed to fill the culture plate with dense, cottony, aerial mycelium, first white, which then became gray. *C. albicans* appeared white in color. *A. niger* was observed as powdery masses of numerous black dots, and *F. solani* was observed to be white to creamish in color, becoming bluish-brown when sporodochia were present (Table 5).

Microscopic characterization

Microscopic examination of the fungi revealed that mycelia were branched in all the tested fungi, except in *C. albicans*. The hyphae of all the examined fungi were septate, except in *R. stolonifer* and *C. albicans*. Conidiophores were observed to be branched, fusiform, long smooth, and coarse roughened in *P. notatum*, *F. solani*, *A. niger*, and *A. flavus*, respectively. Sporangiophores were observed in *R. stolonifer*. Spores were observed in different colors in all the tested fungi, except in *C. albicans* (Table 5).

Antagonistic activity

A combination of two or more microorganisms in a single culture medium may indicate synergism if all of them can grow on the medium and antagonism when only a single species can grow on the medium.

Our results revealed that *P. notatum* was antagonistic to the multi-drug resistant bacteria, *Staphylococcus aureus*. The fungus inhibited the bacterial growth and tolerance towards fungi. The mean diameter of the inhibition zone was 0.9 cm (Figure. 1). No antagonistic activity was observed in other fungi-bacteria combinations (Table 6).

Figure 1. Antagonism between *Staphylococcus aureus* and *Penicillium notatum*

Table 6. Antagonistic activity of the isolated fungal strains

Bacterial combination	Strain antagonistic activity	Growth
Aspergillus flavus + *S. aureus* and *E. Coli*	-ve	No inhibition of growth was observed.
Aspergillus niger + *S. aureus* and *E. Coli*	-ve	No inhibition of growth was observed.
Penicillium notatum + *S. aureus* and *E. Coli*	+ve and -ve	*S. aureus* growth was inhibited by *Penicillium notatum,* but *E. coli* growth was not.
Rhizopus stolonifer + *S. aureus* and *E. Coli*	-ve	No inhibition of growth was observed.
Fusarium solani + *S. aureus* and *E. Coli*	-ve	No inhibition of growth was observed.
Candida albicans + *S. aureus* and *E. Coli*	-ve	No inhibition of growth was observed.

DISCUSSION

A total of 35 fungal isolates were isolated from both, the internal organs and the external surface of *Cx. pipiens* developmental stages and from its breeding environment. These isolates were definitely identified as six fungal strains, namely *A. flavus*, *A. niger*, *P. notatum*, *R. stolonifer*, *F. solani*, and *C. albicans*. These fungi are known to be widely spread in nature, and the most frequent genera were *Aspergillus* and *Penicillium*.

Many authors have isolated fungi from insects (11, 12, 22, 26-33). Other authors have studied the association of fungi with mosquitoes (4, 10, 24, 34, 35).

Agreeable results were presented by Burnside (11) who isolated *A. flavus* and *A. niger* from bees; Gillian and Prest (12) who isolated various fungi from bees and identified the species, *A. niger* and *A. flavus*; Costa and Oliveira (4) who isolated various species of *Penicillium* from the mosquitoes of tropical regions as well as from the mosquitoes of Brazil; Kaaya and Okech (27) who reported various species that were isolated from the pupal and adult *Glossina pallidipes*, including *A. flavus*, *A. niger*, and *Penicillium* spp.; and Zarrin et al. (23) who identified *Aspergillus*, *Penicillium*, and yeast fungi from the external surface of house flies in Ahwaz, Iran.

Other fungal strains were isolated from insects. *Penicillium corylophilum*, *Cladosporium ladosporoides*, and *Alternaria* spp. were isolated from bees (11,12); *Fusarium* species were isolated from larvae and adult insects (26); *Spiroplasma sabaudiense* sp. was isolated from mosquitoes (36); the new *Spiroplasma taiwanense* sp. was isolated from the *Culex tritaeniorhynchus* mosquitoes in Taiwan (34); *Metarhizium anisopliae* and *Beauveria bassiana* were commonly found on terrestrial insects (37); *Cladosporium* and *Fusarium* were isolated from the external surface of house flies (23); *Alternaria* spp., *Penicillium oxalicum*, and *Aspergillus tamari* were isolated from the house fly (*Musca domestica* L.) larvae; and other species, such as *Neurospora* sp., *Alternaria* spp., and *Fusarium oxysporium* were isolated from the larvae of the sheep blow fly, *Lucilia cuprina (*29,33).

Six fungal strains (*A. flavus*, *P. notatum*, *R. stolonifer*, *A. niger*, *C. albicans*, and *F. solani*) were isolated and re-characterized using macroscopic and microscopic properties. These results indicated that mosquito eggs, larvae, pupae, and adults were exposed to fungal infection, either through oral or dermal contamination. However, among these fungi, *F. solani* and *A. niger* showed negative contamination in all the mosquito stages (isolated from the environment, but not in the mosquito developmental stages). It was not clear if transstadial transmission from larvae and pupae to the adult stage had occurred or not; hence, it needs further confirmatory study.

It is well-known that microbes may compete with each other for nutrients, and this competition often results in the inhibition of growth among one of them. This phenomenon is called antagonism (38). In our study, the growth of *P. notatum* inhibited the growth of *S. aureus*. This is due to the secretion of penicillin, which is a well-known antibacterial agent (39). Contrary to our results, Harriott and Noverr (40) reported the antagonism between *S. aureus* and *C. albicans*. However, bacteria or fungi may also rely on other species for nutrient supply.

CONCLUSION

In conclusion, the present study described the isolation of six fungal species from the external surface and the internal organs of the *Cx. pipiens* mosquito developmental stages, and from its breeding environment, as well. Our study demonstrated that the mosquito, *Cx. pipiens*, could harbor many fungal spores, either internally or externally, during its developmental stages. Subsequently, this mosquito will be incriminated in the mechanical dissemination, circulation, and transmission of these fungi in nature, during its life cycle.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethical committee of Cairo University.

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

Authors' Contributions: Conceived and designed the experiments or case: FHG, AMS. Performed the experiments or case: AMS, FHG, OZ, AA. Analyzed the data: FHG, AMS, AA, IA. Wrote the paper: AMS, FHG, IA, AA. All authors have read and approved the final manuscript.

Acknowledgements: The authors thank to the Cairo University and Helwan University for partially contributing some facilities to complete this work as well as all Seufi's laboratory members for their technical support and helpful discussions.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. Southgate BA. Bancroftian filariasis in Egypt. Trop Dis Bull 1979; 76: 1045-68.
2. Gad AM, Farid HA, Ramzy RR, Riad MB, Presley SM. Host feeding of mosquitoes (Diptera: Culicidae) associated with the recurrence of Rift Valley of fever in Egypt. J Med Entomol 1999; 36(6): 709-14. [CrossRef]
3. Lichtwardt RW. The Trichomycetes: Fungal Associates of Arthropods, Springer-Verlag, New York, 1986; pp. 343. [CrossRef]
4. Costa GL, Oliveira PC. Penicillium species in mosquitoes from two Brazilian regions. J Basic Microbiol 1998; 38: 343-47. [CrossRef]
5. Moore GE. Mortality factors caused by pathogenic bacteria and fungi of the southern pine beetle in North Carolina. J Invertebr Pathol 1971; 17: 28-37. [CrossRef]
6. Agarwala SP, Sagar SK, Sehgal SS. Use of mycelial suspension and metabolites of *Paecilomyces lilacinus* (Fungi: Hyphomycetes) in control of *Aedes aegypti* larvae. J Commun Dis 1999; 31: 193-6.
7. Lucarotti CJ, Shoulkamy MA. *Coelomomyces stegomyiae* infection in adult female *Aedes aegypti* following the first, second, and third host blood meals. J Invertebr Pathol 2000; 75: 292-5. [CrossRef]
8. Moraes AM, Corrado M, Holanda VL, Costa GL, Ziccardi M, Lourenco deoliveira R, et al. *Aspergillus* from Brazilian mosquitoes - I. Genera *Aedes* and *Culex* from Rio de Janeiro State. Mycotaxon 2001; 78: 413-22.
9. Scholte EJ, Knols BG, Samson RA, Takken W. Entomopathogenic fungi for mosquito control: A review. J Insect Sci 2004; 4(19): 1-24. [CrossRef]
10. Pereira ES, Ferreira RL, Hamada N, Lichtwardt RW. Trichomycete fungi (Zygomycota) associated with mosquito larvae (Diptera: Culicidae) in natural and artificial habitats in Manaus, AM Brazil. Neotrop Entomol 2005; 34: 325-29. [CrossRef]
11. Burnside CE. Fungus diseases of the honey bee. Bull Technical US Dept Agric 1930; 149: 149-279.
12. Gilliam M, Prest DB. Fungi isolated from the intestinal contents of foraging worker honey bees, *Apis mellifera*. J Invert Pathol 1972; 20: 101-3. [CrossRef]
13. Gilliam M, Prest DB, Morton HL. Fungi isolated from honey bees, *Apis mellifera*, Fed 2,4-D and antibiotics. J Invert Pathol 1974; 24: 213-17. [CrossRef]
14. Zoberi MH, Grace K. Fungi associated with Subterranean Termite *Reticulitermes flavipes* in Ontario. Mycologia 1990; 82: 289-94. [CrossRef]
15. Ismail MA, Abdel-sater MA. Fungi associated with Egyptian cotton *leafworm Spodoptera littoralis* Boisdoval. Mycopathol 1993; 124: 79-86. [CrossRef]
16. Hu X, Li M, Chen H. Community structure of gut fungi during different developmental stages of the Chinese white pine beetle (*Dendroctonus armandi*). Sci Rep 2015; 5: 8411. [CrossRef]
17. Moraes AM, Junqueira AC, Giordano CM. Aspergillus from the digestive tract of Brazilian triatomids. Mycotaxon 1998; 66: 231-241.
18. Moraes AML, Junqueira ACV, Costa GL, Celano V, Oliveira PC, Coura JR. Fungal flora of the digestive tract of 5 species of triatomines vectors of *Trypanosoma cruzi*, Chagas, 1909. Mycopathol 2001; 151: 41-8. [CrossRef]
19. Lage-Moraes AM, Reis-de-Figueiredo A, Vieira-Junqueira AC, Larada-Costa G, Aguiar RK, Cunha-de-Oliveira P. Fungal flora of the digestive tract of Panstrongylus megistus (Reduviidae) used for experimental xenodiagnosis of Trypanosoma (Schizotripanum) cruzi Chagas, 1909. Rev Iberoam Micol 2001; 18: 79-82.
20. Marti GA, García JJ, Cazau MC, López lastra CC. Fungal flora of the digestive tract of *Triatoma infestans* (Hemiptera: Reduviidae) from Argentina. Bol Soc Argent Bot 2007; 42: 175-79.
21. Galal FH, Abu elnasr A, Abdallah I, Seufi AEM, Zaki O. Isolation and Characterization of Internal Bacteria from the Mosquito, *Culex pipiens* from Egypt. Inter J Sci Res 2015; 4: 2682-8.
22. Ozdal M, Incekara U, Polat A, Gur O, Kurbanoğlu EB,Tasar GE. Isolation of filamentous fungi associated with two common edible aquatic insects, *Hydrophilus Piceus* and *Dytiscus Marginalis*. J Bio Micro 2012; 1: 95-105.
23. Zarrin M, Vazirianzadeh B, Solary SS, Mahmoudabadi AZ, Rahdar M. Isolation of fungi from Housefly (*Musca Domestica*) in Ahwaz, Iran. J Med Sci 2007; 6: 917-9.
24. Perira SE, Sarquis MM, Ferreira-keppler LR, Hamada N, Alencar BY. Filamentous Fungi Associated with Mosquito Larvae (Diptera: Culicidae) in Municipalities of the Brazilian Amazon. J Neotr Entomol 2009; 3: 352-9. [CrossRef]
25. Arx JA. The genera of fungi sporulating in pure culture. 3rdedn. J. Cramer, Berlin 1981; pp. 387.
26. Claydon N, Grove F. *Fusarium* as an insect pathogens. In Moss M.O and Smith E, (eds). The Applied Mycology of *Fusarium*. Cambridge University Press. 1984; pp. 115-23.
27. Kaaya GP, Okech MA. Microorganisms associated with tsetse in nature: preliminary results on isolation, identification and pathogenicity. Insect Sci Applic 1990; 11: 443-8. [CrossRef]
28. Banjo AD, Lawal OA, Adeduji OO. Bacteria and fungi isolated from house fly (*Musca domestica* L.) larvae. Afr J Biotechnol 2005; 4: 780-4.
29. Kubatova A, Dvorak L. Entomopathogenic fungi associated with insect hibernating in underground shelters. Czech Mycol 2005; 57: 221-37.
30. Tajedin L, Hashemi J, Abaei M, Hosseinpour L, Rafei F, Basseri H. Study on Fungal Flora in the Midgut of the Larva and Adult of the Different Populations of the Malaria Vector *Anopheles stephensi*. Iranian J Arthropod-Borne Dis 2009; 3(1): 36-40.
31. Shimazu M, Takatsuka J. *Isaria javanica* (anamorphic Cordycipitaceae) isolated from gypsy moth larvae, *Lymantria dispar* (Lepidoptera:Lymantriidae), in Japan. Appl Entomol Zool 2010; 45: 497-504. [CrossRef]
32. Assaf LH, Haleem RA, Abdullah SK. Association of Entomopathogenic and Other Opportunistic Fungi with Insects Dormant Locations. Jordan J Biol Sci 2011; 4: 87-92.
33. Srivoramas T, Chaiwong T, Sanford MR. Isolation of fungi from adult house fly; *Musca Domestica* and the blow fly *Chrysomya Megacephala* in ubon Ratchathani Province, Northeastern ThaiLand. Inter Para J 2012; 4: 53-6. [CrossRef]
34. Abalain-colloc ML, Rosen L, Tully JG, Bove JM, Chastel C, Williamson DL. *Spiroplasma taiwanense* sp. nov. from *Culex tritaeniorhynchus* mosquitoes collected in Taiwan. Int J Syst Bacteriol 1988; 38: 103-7. [CrossRef]
35. Kumar R, Chattopadhyay A, Maitra S, Paul S, Banerjee PK. Isolation and Biochemical Characterizations of Mid Gut Microbiota of *Culex (Culex quinquefasciatus)* Mosquitoes in Some Urban Sub Urban & Rural Areas of West Bengal. J Entomol 2013; 1: 67-78.
36. Abalain-Colloc ML, Chastel C, Tully JG, Bove JM, Whitcomb RF, Gilot B, et al. Spiroplasma sabaudiense sp. nov. from Mosquitos Collected in France. Int J Syst Evol Microbiol 1987; 37: 260-5.
37. Alves SB, Alves LFA, Lopes RB, Pereira RM, Vieira SA. Potential of some Metarhizium anisopliae isolates for control of Culex quinquefasciatus (Dipt. Culicidae). J App Entomol 2002; 126: 504-9. [CrossRef]
38. Talaro K, Talaro A. Foundations in the Microbiology. 4th. Ed. McGraw Hill, New York 2002; pp. 544-52.
39. Fleming A. On the Antibacterial Action of Cultures of a Penicillium, with Special Reference to their Use in the Isolation of B. influenzae. Br J Exp Pathol 1929; 10(3): 226-36.
40. Harriott MM, Noverr MC. Ability of Candida albicans Mutants to Induce Staphylococcu aureus Vancomycin Resistance during Polymicrobial Biofilm Formation. Antimicrob Agents Chemother 2010; 54: 3746-55. [CrossRef]