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Original Research Article

Isolation and Identification of Some Fungi Associated with Both Mature and Immature Stages of Two Mosquito Species

Majida Mohammad Abid Falhy[1]*
[1]University of AL-Qadisiya, College of Science, Department of Biology, Diwaniyah, Iraq

***Corresponding Author**
Majida Mohammad Abid Falhy
University of AL-Qadisiya, College of Science, Department of Biology, Diwaniyah, Iraq

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Abstract: The current study involved isolating two types of fungi associated with mosquito larvae: *Aedes aegypti* and *Culex molestus*, which have not previously been recorded in larval infestations. These fungi were used as biological control agents various developmental stages of mosquitoes *Culex molestus* and *Aedes aegypti* over different time periods (24, 48, 72, 120 hours). The fungi *Lagenidium giganteum* and *Beauveria bassiana* were isolated from naturally infected mosquito larvae and identified in the laboratory. In the pupal stage, the LC_{50} values were (7.411×10^6, 7.373×10^6) spore/ml for *Lagenidium giganteum* in *Aedes aegypti* and *Culex molestus*, respectively, after 72 hours of treatment. While the LC_{50} values for *Beauveria bassiana* were (9.473×10^6, 9.371×10^6) spore/ml for the two mosquito species after the same time period. This indicates the superiority of *Lagenidium giganteum* in achieving higher mortality rates compared to *Beauveria bassiana.* Regarding adults, female mosquitoes exhibited greater resistance compared to males. Additionally, males and females of *Culex molestus* were more sensitive to the fungal suspensions than to *Aedes aegypti*. LC_{50} values for females of *Aedes aegypti* and *Culex molestus* were (9.108×10^6) and (6.159×10^6) spore/ml, respectively, after 72 hours.

Keywords: Culex Molestus ‹Aedes Aegypti‹ Beauveria Bassiana, Lagenidium Giganteum.

INTRODUCTION

Mosquitoes (Family: Culicidae) pose a significant threat to global health as they are efficient vectors of major infectious agents [1, 2]. The mosquito genera of medical importance are Anopheles, Culex, and Aedes, which are the most efficient pathogen vectors of the class of Arthropods [3, 4] , carry pathogens (parasites, filarial worms, and arboviruses) which are responsible for at least 17% of all human and animal diseases [5, 6].

Mosquitoes are major disease vectors found on all continents except Antarctica. They are significant from both medical and economic perspectives due to their role in transmitting various diseases such as anemia, itching, allergies, and general nuisance. *Culex molestus* is unique because it can mate in confined spaces without the need to fly in swarms, a phenomenon known as "Steno gamy." Additionally, females are capable of laying their first batch of eggs without a blood meal, a condition referred to as Autogeny [7].

Researchers have been interested in mosquito control for a long time, with chemical control methods being the most effective in reducing mosquito populations and their associated problems globally. However, the use of synthetic pesticides has

caused significant environmental damage, including air, water, and soil pollution, which leads to changes in the quality of these vital environmental components. Additionally, targeted insects have rapidly adapted to toxic substances, developing resistance to them [8]. Various plant extracts and insect growth regulators have also been used for mosquito control, but these methods have not achieved the desired results due to the mosquitoes' ability to adapt and develop resistance. Consequently, researchers have been exploring alternative methods. Pathogenic fungi are considered significant biological control agents due to their widespread presence in nature, low cost, and high specificity against particular pests [9]. Thus, attention has turned to using fungal suspensions for insect control as a safer alternative to synthetic pesticides, given that these fungi can be toxic, anti-feeding, or inhibitory to insects [10].

Among these fungi are those belonging to the genera *Beauveria* and *Lagenidium*. Some of these fungi can infect mosquitoes under suitable conditions for spore development. *Lagenidium giganteum*, an Oomycete fungus, parasitizes many mosquito larvae of the genera *Culex* and *Anopheles*. Infection occurs through fungal spores from the digestive tract and body wall of the host. The pathogenic stage of this fungus is the biflagellate swimming spore.

Beauveria bassiana, an Ascomycete fungus, is known for its pathogenicity in insects. It has been noted for its effects on whiteflies (*Bemisia sp.*) and scale insects [11]. Considering the medical importance of mosquitoes, the aim of this study to isolate fungi from naturally infected larvae of *Culex molestus* and *Aedes aegypti* and evaluate their effectiveness as alternatives to chemical pesticides. The study included:

1. **Isolation and Identification some Pathogenic Fungi**: Isolate and identify pathogenic fungi from the larvae of *Culex molestus* and *Aedes aegypti* in local environments and various areas in Al-Diwaniyah city. This involved preparing suitable media for isolation in the laboratory, cultivating and propagating the fungi, and calculating the percentage of occurrence of the isolated fungi.
2. **Fungal Growth Evaluation**: Cultivate the fungi on various growth media over different time periods to determine the optimal duration for growth.
3. **Effectiveness of Fungal Suspensions**: Assess the impact of different concentrations of fungal suspensions on the mortality rates of various life stages (egg, larva, pupa, adult) of *Culex molestus* and *Aedes aegypti*.

MATERIALS AND METHODS

Isolation and Identification of Fungi Associated with Mosquito Larvae

To isolate pathogenic fungi, infected and dead mosquito larvae showing signs of fungal infection (e.g., discoloration and fungal mycelium on the larval body) were used. The larvae were sterilized by immersion in 70% ethanol for 10 seconds, then transferred to 5% sodium hypochlorite solution for 2 minutes, and finally washed by distilled water, then placed on filter paper, then transferred with sterile tweezers to plastic Petri dishes containing media for fungal isolation. The dishes were incubated at 25 ± 2°C for 7 days [12].

To purify the isolated fungi, a 0.5 cm disk from the edge of the growing fungal colony was transferred with a sterile cork borer to the center of a Petri dish containing solid media. The dishes were incubated under the same conditions for 7 days. Fungal identification was performed microscopically by placing a small part from the fungal growth on a glass slide with a drop of sterile distilled water, covering it with a coverslip, and examining it under a light microscope. Fungi were identified using taxonomic keys [13, 14].

Establishment of Permanent Cultures of *C. Molestus* and *A. Aegypti*

Different developmental stages of larvae from both mosquito species were collected from water drainage sites in Al-Diwaniyah province using a long-handled scoop. They were placed in plastic bottles with lids and transported to the laboratory. Then placed in plastic containers (30 x 15 cm) filled with dechlorinated water and fed with ground mouse food composed of (wheat, corn, protein and, rice) in a 1:1:1:0.25 ratio, with 2 grams per container. The containers were covered with mesh fabric.

To establish a permanent, pure culture, newly emerged pupae of *C. molestus* were transferred using a wide-mouthed pipette to plastic containers, which were then placed in a cube-shaped cage (50 cm per side) covered with mesh fabric. Inside the cages, Petri dishes with cotton saturated with a 10% sugar solution were placed. To obtain egg rafts, the method of [15], was followed. Female mosquitoes were fed with pigeon blood three days after emergence. Their wings were clipped, legs were tied, and they were placed over the rearing cage overnight. A small water container was placed in the cage for egg laying. Eggs were transferred with a small brush to new water containers with larval food and monitored until adult emergence, taking care to avoid spoilage by changing the water every three days. This process was repeated until the third generation.

To ensure sufficient numbers of each stage, enough eggs were isolated to obtain 1st larval stage. For the second, third, and fourth larval stages, adequate numbers of larvae from the preceding stage were isolated and placed individually in rearing tubes to be monitored until they molted to the desired stage for testing.

Picture 1: Swamps from mosquito culture were collected

Preparation of Fungal Suspensions of *L.Giganteum* and *B.Bassiana*

The fungal suspension for each fungus was prepared by adding 5 ml of sterile distilled water to a 14-day-old fungal culture on PDA medium in 9 cm Petri dishes. Tween-80 was added at a concentration of 0.01% as a surfactant. Spores were harvested using a sterile glass rod, and the suspension was mixed using a magnetic stirrer for 10 minutes. The contents were filtered by funnel with a sterile cheesecloth, and an additional 5 ml from distilled water was added to ensure complete filtration of the fungal spores. The filtrate, totaling 10 ml, was collected in a glass flask and considered the stock suspension [16].

To count the spores, 1 ml from the filtrate was placed on a hemocytometer slide to estimate the number of spores per unit volume. The spores was averaged, and the result was multiplied by 1×10^4 to obtain the spore count per ml. The concentrations were:

- *Lagenidium giganteum*: 3×10^6 spores/ml
- *Beauveria bassiana*: 2×10^6 spores/ml

To prepare lower concentrations, the following formula was used [17]:

- Volume (ml) of stock suspension=Desired con centration/Basic suspension concentration.

The result was multiplied by the volume of suspension. Thus, the concentrations were prepared as:

- *Lagenidium giganteum*: 3×10^5, 3×10^4, 3×10^3, 3×10^2 spores/ml
- *Beauveria bassiana*: 2×10^5, 2×10^4, 2×10^3, 2×10^2 spores/ml

Study of the Effects of Different Fungal Suspension Concentration on Mortality Rates of *Aedes Aegypti* and *Culex Molestus* Life Stages

Effect on Egg Mortality Rates

Egg rafts, 24 hours old, were collected from *C. molestus* or individual eggs from *A. aegypti* (100 eggs per replicate) and placed in plastic containers which contain 100 ml of each concentration from fungal suspensions. The eggs were also sprayed with the same concentration of fungal suspension from a height of 15 cm to ensure exposure. Control treatments were sprayed with sterile distilled water only. Containers were incubated at 25 ± 2°C, and egg mortality rates were recorded until hatching [18].

Effect on Larval Mortality Rates

Forty larvae from each larval stage were placed in containers, three contain100 ml of each concentration of the tested fungal suspensions, and one containing distilled water (control). Treated larvae were transferred to glass containers by distilled water and fed with 10 mg of larval food. The containers were incubated at 25 ± 2°C with a 14/10-hour light/dark cycle. Mortality rates were recorded at 24, 72, and 120-hours post-treatment [19].

Effect on Pupal Mortality Rates

Pupae of *C.molestus* and *A.aegypti* were isolated from fourth-stage larvae, and the same procedure was followed as for larvae, except no larval food was added. Containers were covered with mesh fabric to prevent adult emergence. Mortality rates were recorded at 24, 48, and 72 hours [20].

The Effect on the Mortality Percentages of the Adults

Sufficient numbers of pupae from each species were collected individually from the permanent farm and placed in 10 ml tubes, which were sealed with some cotton until they turned to mature. Then, glass beakers with a capacity of 1 liter each were prepared, and each contained a piece of cotton soaked in a 10% sugar solution placed in a small dish. Each beaker was sprayed with 5 ml of the fungal suspensions using a manual sprayer from a height of approximately 15 cm, while the control treatment was sprayed with distilled water. Afterward, 10 newly emerged adults from both male and female of each species were transferred separately into the treated beakers using a pipette. This experiment was repeated three times for each concentration and similarly for the control treatment. Then incubated under the same conditions, and the mortality percentage was recorded daily for (7) days [21].

Statistical Analysis

Data were statistically analyzed by SPSS software with a completely randomized design (C.R.D) for factorial and single-factor experiments. Percentage data were arcsine transformed and means were compared using the Revised Least Significant Difference (R.L.S.D) at a significance level of ($P \leq 0.05$) [22]. Mortality percentages were corrected using [23].

RESULTS AND DISCUSSION

Effect of Fungal Suspensions of *Beauveria Bassiana* and *Lagenidium Giganteum* on the Mortality Percentage of *Aedes Aegypti* and *Culex Molestus* Eggs.

Table (1) shows the results of the effect of different concentrations of fungal suspensions of *Beauveria bassiana* and *Lagenidium giganteum* on the mortality percentages of *Culex molestus* and *Aedes aegypti* eggs. It was observed that *Lagenidium giganteum* outperformed *Beauveria bassiana* at all concentrations. A positive correlation was noted between the concentrations and the mortality percentage, meaning that mortality increased with higher concentrations.

Statistical analysis revealed significant differences between the fungal suspension concentrations, which is clearly reflected in the LC_{50} values. The LC_{50} values for *Lagenidium giganteum* suspension were (4.874×10^6, 4.484×10^6), while for *Beauveria bassiana* suspension, the LC_{50} values were (6.094×10^6, 5.566×10^6) spores/ml. This indicates that *Aedes aegypti* eggs were more sensitive to the fungal suspension than *Culex molestus* eggs.

The ability of these fungi to penetrate the egg shell is attributed to the combination of enzymatic and mechanical activities, as they can secrete protease, chitinase, and lipase enzymes, as well as exert mechanical action [24, 25], reported that exposure of whitefly eggs to *Beauveria bassiana* suspension caused 81.1% mortality after seven days of exposure. Similarly [26], confirmed that exposure of *Rhipicephalus annulatus* tick eggs to *Beauveria bassiana* at 1×10^1 spores/ml had no significant effect on hatching percentage [27], observed that exposure of *Aedes aegypti* eggs to *Metarhizium anisopliae* spores at a concentration of 2.8×10^2 spores/ml reduced the hatching percentage to 50% [28], indicated that exposure of *Cx. pipiens* eggs to *Beauveria bassiana* spores led to 100% mortality.[29] showed that treatment of *Cx. quinquefasciatus* and *An. pulcharhimus* eggs with *Lagenidium lundbergii* at 3×10^7 spores/ml resulted in 56% and 59.33% mortality, respectively [30], reported that the hatching percentage of *Cx. pipiens* eggs exposed to *Metarhizium anisopliae* at 3×10^3 spores/ml decreased to 40% [31], also observed a reduction in the hatching percentage of *Cx. quinquefasciatus* eggs by 59% when exposed to *C. keratinophilum* spores at a concentration of 2×10^6 spores/ml. These results

were consistent with [32], who found that exposure of *Cx. quinquefasciatus* eggs to *P. marneffei* spores resulted in an increased mortality rate with higher concentrations, reaching 46.03% at 2×10^4 spores/ml.

This overall data emphasizes the potential use of fungal suspensions as biocontrol agents to manage mosquito populations by targeting their eggs.

Table 1: LC_{50} and LC_{90} Values for fungal suspensions against *Culex molestus* and *Aedes aegypti* eggs

LC	*A.aegypti*		*C.molestus*	
	L.giganteum	***B.bassiana***	***L.giganteum***	***B.bassiana***
LC_{50} value	4.874 $x10^6$	6.094 $x10^6$	4.484$x10^6$	5.566$x10^6$
Limits 95%	1.983 x 10^6- 7.524 x 10^7	3.754$x10^6$- 2.202$x10^7$	1.964$x10^6$- 5.642$x10^6$	2.633$x10^6$- 5.782$x10^6$
LC_{90} value	1.490 x 10^7	1.539$x10^7$	1.850x107	1.585$x10^7$
Limits 95%	8.538 $x10^6$- 4.965 $x10^7$	9.138$x10^6$- 5.977$x10^7$	1.106$x10^7$- 6.170$x10^7$	9.742$x10^6$- 5.997$x10^7$
X2	3.811	2.401	3.227	2.099
P value	0.149	0.301	0.199	0.350
Regression equation	Y=0.63+1.31 E -7*X	Y=-0.85+1.41E-7*X	Y=-0.41+9.31E-7*X	Y=-07+1.27E-7*X

Tables (2 and 3) illustrate the effect of various concentrations of fungal suspensions being investigated on the percentage mortality of the four larval stages of *A. aegypti* and *C. molestus* mosquitoes. The suspension of *L. giganteum* was found to be more effective at all concentrations compared to *B. bassiana*.

To measure the pathogenicity, LC_{50} and LC_{90} values were calculated, which are fundamental in testing methods [33]. These values increase proportionally with the progression of the larval stage. Additionally, the P-value and regression equation were calculated. The regression equation is a statistical formula representing the relationship between two variables and is used to estimate past values and predict future values, helping to describe the relationship between variables. The LC_{50} values for *B. bassiana* suspension were 1.165×10^6, 2.944×10^6, 3.337×10^6, and 4.561×10^6 spore/ml for *A. aegypti*, while they were 5.241×10^6, 1.845×10^6, 1.868×10^6, and 4.45×10^6 spore/ml for *C.molestus* after 120 hours of treatment for *L.giganteum* suspension, the LC_{50} values were 1.163×10^6, 2.880×10, 3.146×10^6, and 4.206×10^6 spore/ml for *A. aegypti*, and 1.671×10^5, 7.1×10^5, 7.262×10^5, and 1.777×10^6 spore/ml for *C.molestus* after the same time period.

Regarding sensitivity across larval stages, LC_{50} values confirmed that the first larval stage was the most sensitive to the tested fungal suspensions compared to the other stages, showing the lowest LC_{50} values. The *A.aegypti* mosquitoes were more sensitive compared to *C. molestus*. This result is consistent with [34], who found that first and second instar larvae of *Anopheles stephensi* treated with *Chrysosporium evolceanui* suspension had LC_{50} values of 1.1×10^3 and 1.4×10^3 spore/ml, respectively. The results also align with [35], who reported LC_{50} values of 8×10^3, 3.9×10^7, 7.5×10^6, 6×10^7, and 5.1×10^5 spore/ml for fourth instar larvae of *Culex pipiens* exposed to *Metarhizium anisopliae* at 24, 48, 72, and 96 hours. In another study [36], found an LC_{50} value of 3×10^5 spore/ml for third instar larvae of *Culex quinquefasciatus* exposed to *Penicillium citrinum* suspension at a concentration of 1×10^6 spore/ml [37], found that for third instar larvae of *Anopheles stephensi* and *Culex quinquefasciatus* treated with *Metarhizium anisopliae* at a concentration of 1×10^6 spore/ml, the LC_{50} values were 1×10^4 and 9.2×10^5 spore/ml, respectively [38], found that LC_{50} values for the four larval stages of *Culex quinquefasciatus* treated with *Beauveria bassiana* were 3.58, 5.84, 6.95, and 11.53 mg/l, respectively. For third instar larvae of *Anopheles stephensi* and *Culex quinquefasciatus* treated with *Fusarium oxysporum*, the LC_{50} values were 109.24 and 320.30 mg/L, respectively, after 24 hours [39]. For *Anopheles sp* larvae exposed to *Trichoderma asperellum*, the LC_{50} value was 2.68×10^7 spore/ml [40].

The increase in mortality percentage with increasing concentration is attributed to the higher number of conidia, leading to a higher proportion of developing conidia when attacking the host and weakening the insect's immune system. Additionally, the immune system of larvae can defend the body at lower concentrations, but at higher concentrations, the immune system may lose its effectiveness [41]. Statistical analysis has shown significant differences in the corrected mortality percentages across the four larval stages, with significant differences observed between the two mosquito species. The four larval stages of *A. aegypti* were more sensitive to all fungal suspensions and concentrations compared to *C. molestus*. The relationship between mortality rates and larval stage age was found to be inverse, possibly due to the incomplete immune system in the early stages and the delicate body tissues, which make it easier for the spores to penetrate [42]. The variation in mortality rates among the studied fungi

may be due to differences in their ability to produce enzymes and toxins that affect the physiological activities of insects, leading to their death [43, 44], found that exposure of *Culex quinquefasciatus* larvae to *Beauveria bassiana*100% mortality at 1×10^8 spore/ml and 97% mortality at1×10^7 spore/ml [31], reported that mortality rates increased with higher concentrations of the fungal suspension of *C. keratinophilum* for all four larval stages of *Culex quinquefasciatus*, which is consistent with findings by [45], who described the relationship between *Aedes aegypti* larval stages and mortality rates. They found that mortality decreased with age, with 100% mortality in the first and second instar larvae and 40% in the third and fourth instars when treated with *L. chapmani* suspension at a concentration of 3.65×10^5 spore/ml [46], found that for third and fourth instar larvae of *Anopheles stephensi* and *Anopheles gambiae* treated with *Metarhizium anisopliae* suspension, mortality was lower in the third and fourth instars compared to the first and second instars for both mosquito species. Infected insects may live 3 to 5 days due to spore germination and fungal hyphae penetration through respiratory openings, which causes larvae to suffocate as respiratory openings become blocked. Additionally, fungal growth in the larval midgut depletes nutrients, and after 72 hours, fat tissues break down, potentially leading to 100% larval mortality after 96 hours. Some larvae die through molting as they fail to molt and stay attached to the old cuticle [47, 48], added that ingestion of spores by larvae is followed by toxin secretion from the fungi, leading to blood poisoning. The high mortality rates in early larval stages compared to adults and late larval stages are attributed to the incomplete defensive cells in early larval stages, especially the first instar, and the thinner cuticle. It might also be explained by chemical and biological changes in the body wall, such as the presence of toxic compounds that may prevent spore germination in later stages [49].

The results of this study were similar to those found by [50], who observed a significant effect of the fungi *M. anisopliae* and *B. bassiana* on the mortality rates of both complete and incomplete life stages of *Tribolium castaneum*. The fungi's ability to adhere to the insect body, form germ tubes, attachment organs, and the quantity of enzymes such as chitinase, lipase, and protease produced played a significant role in insect body degradation.

The study by [51], showed susceptibility in *Culex molestus* strains to plant extracts and the Mozkill insecticide after three generations. LC_{50} values were 0.72, 0.77, and 0.20 mg/ml for cold water, boiled, and hexane extracts of ash plant, respectively, and 1.05, 0.91, and 1.15 mg/ml for cold water, boiled, and hexane extracts of eucalyptus. The LC_{50} value for Mozkill was 1.01 mg/ml, with the strains showing greater sensitivity to ash plant extracts compared to eucalyptus extracts and Mozkill. *Anopheles gambiae* larvae (3rd and 4th instar) were exposed to five different concentrations of *L. giganteum* and *L. ajelloi* zoospores; 1000, 2000, 3000, 4000, and 5000 zoospores/mL, respectively,the larval mortality was recorded after 24, 48, 72, and 96 hours post-exposure, until all larvae were dead. The results obtained showed that *L. giganteum* was not pathogenic to *Anopheles gambiae* larvae after 24 and 48 hours post-exposure to all concentrations. Larval mortality was recorded at 72 and 96 hours. The highest concentration 5000 zoospores/mL) of *L. giganteum* tested against *Anopheles gambiae* larvae killed 68% of the exposed larvae in 96 hours [52]. However, these results differed slightly from those of [53], who found that 56% of *A. gambiae* larvae exposed to *L. giganteum* zoospores were protected from death by the larval immune defense [52, 53]. Both findings support the fact that the pathogenicity of *L. giganteum* to *A. gambiae* increases with zoospore concentration and those different strains of the fungus may produce different virulence and pathogenicity, the weak pathogenicity of *L. giganteum* zoospores observed in the study can also be attributed to their inability to sometimes recognize late instars of otherwise susceptible mosquito larvae.

Table 2: LC_{50} and LC_{90} values for the bioassay of *L. giganteum* Suspensions in the four larval stages of *C. molestus* and *A. aegypti*

LC	*A.aegypti*												*C.molestus*											
	1 instar			2 instar			3 instar			4 instar			1 instar			2 instar			3 instar			4 instar		
	24	72	120	24	72	120	24	72	120	24	72	120	24	72	120	24	72	120	24	72	120	24	72	120
LC_{50} value	1.360×10^7	3.962×10^6	1.163×10^6	1.387×10^7	4.235×10^6	2.880×10^6	1.078×10^7	7.675×10^6	3.146×10^6	1.109×10^7	7.432×10^6	4.206×10^6	6.333×10^6	2.928×10^6	1.671×10^5	8.480×10^6	3.962×10^6	7.1×10^5	9.373×10^6	5.425×10^6	7.262×10^5	1.078×10^7	5.375×10^6	1.777×10^6

LC	*A.aegypti*												*C.molestus*											
	1 instar			2 instar			3 instar			4 instar			1 instar			2 instar			3 instar			4 instar		
	24	**72**	**120**	**24**	**72**	**120**	**24**	**72**	**120**	**24**	**72**	**120**	**24**	**72**	**120**	**24**	**72**	**120**	**24**	**72**	**120**	**24**	**72**	**120**
Limits 95%	1.124x107-1.537x10^7	2.813X10^6-4.352X10^6	1.131X10^6-1.742X10^6	9.372X10^6-1.702X10^7	3.128X10^6-5.614X10^6	6.714X10^6-9.974X10^6	8.941X10^6-1.242X10^7	5.821X10^6-9.124X10^6	2.014X10^6-4.682X10^6	9.427X10^6-1.319X10^7	5.712X10^6-9.013X10^6	3.874X10^6-5.314X10^6	4.215X10^6-7.622X10^6	2.014X10^6-3.722X10^6	1.32X10^5-1.93X10^5	7.001X10^6-9.841X10^6	2.813X10^6-4.352X10^6	1.8796-3340X10^4	8.902X10^6-9.942X10^5	4.003X10^6-6.721X10^6	6.112X10^5-8.093X10^5	8.314X10^6-1.371X10^7	4.105X10^6-6.312X10^6	1.124X10^6-2.003X10^6
LC_{90} value	4.011x10^7	1.473X10^7	8.925X10^6	3.476X10^7	1.336X10^7	1.126X10^7	2.584X10^7	2.376X10^7	1.182X10^7	2.507X10^7	2.024X10^7	1.214X10^7	1.765X10^7	1.593X10^7	9.85X10^6	2.210X10^7	1.473X10^7	9.806X10^6	2.337X10^7	1.830X10^7	9.540X10^6	2.584X10^7	1.530X10^7	1.272X10^7
Limits 95%	3.171x10^7-5.231x10^7	8.337X106-1.952X10^7	6.805X10^6-1.092X10^7	2.171X10^7-5.311X10^7	1.013X10^7-1.972X10^7	9.741X10^6-1.371X10^7	1.243X10^7-3.912X10^7	1.782X10^7-3.417X10^7	9.711 X10^6-1.373X10^7	1.714 X10^7-3.213X10^7	1.614X10^7-3.014X10^7	1.032X10^7-1.482X10^7	1.231X10^7-2.413X10^7	1.144X10^7-2.031X10^7	8.13X106-1.131X10^7	3.030X10^7-3.3206410	8.337X106-1.951X10^7	7.846X10^6-1.280X10^7	1.377X10^7-3.501X10^7	1.480X10^7-2.431X10^7	7.84X10^7-1.252X10^7	1.964X10^7-3.484X10^7	1.138X10^7-1.932X10^7	9.735X10^6-1.582X10^7
X2	0.537	0.702	1.517	0.254	1.224	1.024	0.644	0.859	1.036	0.726	0.499	1.665	0.499	0.376	1.507	0.570	0.702	0.680	1.078	0.774	0.982	0.644	0.832	1.537
P value	0.765	0.704	0.468	0.881	0.542	0.599	0.725	0.651	0.596	0.696	0.779	0.435	0.779	0.829	0.471	0.752	0.704	0.712	0.583	0.679	0.612	0.725	0.660	0.464
Regression equation	Y=-0.66+4.95E-7*X	Y=-0.47+1.2E8*X	Y=-0.17+1.23E-7*X	Y=-0.85+6.23E-8*X	Y=-0.6+1.43E-8*X	Y=-0.1+1.23E-7*X	Y=-0.94+8.75E-8*X	Y=-0.62+8.15E-8*X	Y=-0.17+1.23E-7*X	Y=-1.03+9.5E- 8*X	Y=-0.75+1.02E-7*X	Y=-0.32+1.33E-7*X	Y=-0.72+1.15E-7*X	Y=-0.29+9.89E-8*X	Y=-0.02+1.27E-7*X	Y=-0.8+9.6E-8*X	Y=-0.47+1.2E-8*X	Y=-3.54+1.3E-7*X	Y=-0.87+9.52E-8*X	Y=-0.54+1.01E-8*X	Y=-0.1+1.45E-7*X	Y=-0.92+8.75E-8*X	Y=-0.7+1.31E-7*X	Y=-0.21+1.18E-7*X

Table 3: LC_{50} and LC_{90} values for the bioassay of *B. bassiana* Suspension in the Larval Stages of *C. molestus* and *A. aegypti*

LC	*A.aegypti*												*C.molestus*											
	1 instar			2 instar			3 instar			4 instar			1 instar			2 instar			3 instar			4 instar		
	24	**72**	**120**	**24**	**72**	**120**	**24**	**72**	**120**	**24**	**72**	**120**	**24**	**72**	**120**	**24**	**72**	**120**	**24**	**72**	**120**	**24**	**72**	**120**
LC_{50} value	1.425x10^7	4.345X10^6	1.165X10^6	1.464X10^7	1.104X10^7	2.944X10^6	1.475X10^7	8.480X10^6	3.337X10^6	1.476X10^7	9.242X10^6	4.561X10^6	1.207X10^7	2.935X10^6	5.241X10^5	9.362X10^6	5.988X10^6	1.845X10^6	1.464X10^7	6.696X10^6	1.868X10^6	1.465X10^7	8.913X10^6	4.45X10^6
Limits 95%	3.241x10^4-1.132x10^7	1.332X10^5-8.213X10^7	8.311X10^5-1.341X10^6	9.724X10^6-1.611X10^7	3.920X10^7-6.724X10^7	1.768X10^6-2.947X10^6	9.324X10^6-1.389X10^7	7.121X10^6-1.036X10^7	2.158X10^6-4.185X10^6	1.2176110-1.682X10^7	7.113X10^6-1.237X10^7	3.318X10^6-5.883X10^6	3.112X10^6-2.344X10^7	1.544X10^6-5.856X10^5	2.457X10^6-3.106X10^6	1.772X10^6-5.133X10^7	3.722X10^6-9.233X10^6	9.112X10^6-2.131X10^6	1.137X10^7-2.872X10^7	5.123X106-7.692X10^6	1.132X10^6-2.733X10^6	9.321X10^6-2.391X10^7	7.223X10^6-9.824X10^7	3.122X10^6

LC	*A.aegypti*												*C.molestus*											
	1 instar			2 instar			3 instar			4 instar			1 instar			2 instar			3 instar			4 instar		
	24	**72**	**120**	**24**	**72**	**120**	**24**	**72**	**120**	**24**	**72**	**120**	**24**	**72**	**120**	**24**	**72**	**120**	**24**	**72**	**120**	**24**	**72**	**120**
LC_{90} value	2.129x10^7	1.414X10^7	1.002X10^7	3.256X10^7	1.384X10^7	1.495X10^7	2.517X10^7	2.210X10^7	1.329X10^7	3.984X10^7	2.468X10^7	1.500X10^7	2.957X10^7	1.084X10^7	8.540X10^6	1.473X10^7	1.849X10^7	1.248X10^7	3.256X10^7	1.916X10^7	1.235X10^7	3.744X10^7	2.179X10^7	2.094X10^7
Limits 95%	1.827x10^5-6.324x10^7	1.002X10^7-1.812X10^7	8.724X10^6-1.163X10^7	2.051X10^7-4.770X10^7	1.012X10^7-1.541X10^7	1.132X10^7-1.791X10^7	2.034X10^7-3.100X10^7	1.731X10^7-3.142X10^7	1.031X10^7-3.251X10^7	2.114X107-4.628X10^7	1.985X10^6-3.172X10^7	9.221X10^6-2.422X10^7	1.125X10^7-3.742X10^7	5.871X10^6-2.785X10^7	4.598X10^6-6.335X10^7	1.772X10^6-2.462X10^7	1.298X10^6-2.143X10^7	9.723X10^6-1.672X10^7	2.632X10^7-4.426X10^7	1.122X10^7-2.732X10^7	9.224X10^6-2.162X10^7	2.411X10^7-4.322X10^7	1.621X10^6-3.251X10^7	1.322X10^7 -3.314X10^7
X2	0.703	0.779	0.994	0.321	0.989	1.097	0.084	0.570	1.699	0.377	0.211	0.736	0.660	0.633	0.537	0.703	0.818	0.613	0.321	0.176	0.651	0.535	0.627	0.411
P value	0.704	0.677	0.608	0.852	0.610	0.578	0.959	0.752	0.428	0.828	0.900	0.692	0.719	0.729	0.764	0.704	0.664	0.736	0.852	0.916	0.722	0.765	0.731	0.814
Regression equation	Y=-0.99+1.1E-7*X	Y=-0.57+1.32E-8*X	Y=-0.17+1.45E-7*X	Y=-1.05+7.32E-8*X	Y=-0.76+1.5E-8*X	Y=-0.23+1.02E-7*X	Y=-1.12+9.63E-8*X	Y=-0.8+9.6E-8*X	Y=-0.43+1.31E-7*X	Y=-1.17+8.17E-8*X	Y=-0.77+8.38E-7*X	Y=-0.55+1.23E-7*X	Y=-0.89+7.5E-7*X	Y=-0.48+1.63E-8*X	Y=-0.08+1.6E-7*X	Y=-0.47+1.2E-8*X	Y=-0.62+1.04E-8*X	Y=-0.14+1.14E-7*X	Y=-1.05+7.32E-8*X	Y=-0.69+1.03E-8*X	Y=-0.23+1.23E-7*X	Y=-1.13+6.72E-8*X	Y=-0.89+1.12E-7*X	Y=-0.35+7.85E-7*X

Bioassay of *L. Giganteum* and *B. Bassiana* Suspensions on Pupae of *A. Aegypti* and *C. Molestus*

Table (4) shows the impact of various concentrations of *B. bassiana* and *L. giganteum* suspensions on the pupae of *A. aegypti* and *C. molestus*. The results indicate a positive correlation between concentration and mortality rates, as well as between exposure duration and mortality rates.

For *L. giganteum*, the lowest mortality rates were (7.69%, 10.52%, 13.15%) for *A. aegypti* pupae and (10%, 10.25%, 20.51%) for *C. molestus* pupae at the 3×10^2 spore/ml. The highest mortality rates were (15.83%, 21.05%, 28.94%) for *A. aegypti* pupae and (17.5%, 23.07%, 33.33%) for *C. molestus* pupae at 3×10^5 spore/ml. Similarly, the highest mortality rates for *B.bassiana* were 10.52%, 15.87%, 23.68% for *A. aegypti* pupae and (15.38%, 23.07%, 25.64%) for *C. molestus* pupae at the highest concentration of 2×10^5 spore/ml after 24, 48, and 72 hours. These results were statistically supported by significant differences between treatments and mosquito species. *A. aegypti* pupae were more sensitive to fungal suspensions than to *C molestus* pupae. The LC_{50} values for *A. aegypti* and *C. molestus* pupae were (7.411×10^6, 7.373×10^6) spore/ml and (9.473×10^6, 9.371×10^6) spore/ml, respectively, when exposed to *L. giganteum* and *B bassiana* suspensions after 72 hours (Table 4).

The reduced effect of the fungal suspension on pupae is attributed to the cuticle, which is more rigid than in the larval stages due to the higher chitin levels just before adult emergence. This cuticle layer reduces the penetration of pathogenic fungal spores. Additionally, pupae require a shorter period to transform into adults, allowing them to escape the fungal effects [54, 55], observed a 96% mortality rate in *Culex pipiens* pupae when exposed to *Beauveria bassiana* at 5×10^6 spore/ml. [56] found mortality rates ranging from 63% to 88% in *Culex pipiens* pupae when exposed to *E. culicis* suspension [57], reported a 74% mortality rate in *Anopheles stephensi* pupae after 3 days of treatment with *Metarhizium anisopliae* suspension. The effect of *C. keratinophilum* suspension on *Culex quinquefasciatus* pupae caused 50% mortality after three days of treatment [31, 32], found a 47%mortality rate in *Culex quinquefasciatus* pupae when exposed to *Penicillium marnifei*

suspension at 2×10^4 spore/ml. In other studies, testing the effect of *Beauveria bassiana* on *Culex quinquefasciatus* pupae, the LC_{50} value was found to be 9.04×10^5 spore/ml after one day of treatment [38]. The cause of mortality in pupae is attributed to fungal infection leading to the depletion of the internal tissues of the pupa, which prevents successful emergence or causes the insect to die within the pupal case, leading to failed emergence. This finding is consistent with [58], reported when treating *Culex quinquefasciatus* pupae with *Metarhizium anisopliae* suspension.

Table 4: LC_{50} and LC_{90} values of fungal suspension in the pupal stage of *A. aegypti* and *C. molestus*.

LC	*A.aegyptia*						*C.molestus*					
	L.gigainteum			*B.bassiana*			*L.giganteum*			*B.bassianiа*		
	24	48	72	24	48	72	24	48	72	24	48	72
LC_{50} value	$1.637x10^7$	$1.207x10^7$	$7.411x10^6$	$1.731x10^7$	$1.628x10^7$	$9.473x10^6$	$1.632x10^7$	$8.549X10^6$	$7.373X10^6$	$1.264X10^7$	$9.462X10^6$	$9.371X10^6$
Limits 95 %	$1.012x10^7$-$1.671x10^7$	$9.162x10^6$-$1.405x10^7$	$6.121x10^6$-$8.721x10^6$	$1.141x10^7$-$2.084x10^7$	$8.721x10^6$-$1.811x10^7$	$7.921x10^6$-$1.135x10^7$	$9.255X10^7$-$1.948X10^7$	$7.214X10^6$-$9.935X10^6$	$6.133X10^6$-$9.012X10^6$	$1.031X10^7$-$2.937X10^7$	$8.163X10^6$-$1.082X10^7$	$8.143X10^5$-$1.142X10^6$
LC_{90} value	$2.751x10^7$	$2.957x10^7$	$2.018x10^7$	$3.512x10^7$	$3.686x10^7$	$2.337x10^7$	$3.502X10^7$	$1.928X10^7$	$2.223X10^7$	$2.505X10^7$	$2.129X10^7$	$2.656X10^6$
Limits 95 %	$1.320x10^7$-$3.964x10^7$	$1.614x10^7$-$3.867x10^7$	$1.135x10^7$-$3.612x10^7$	$2.105x10^7$-$4.911x10^7$	$2.031x10^7$-$4.831x10^7$	$1.725x10^7$-$3.781x10^7$	$1.953X10^6$-$5.024X10^6$	$9.142X10^6$-$2.472X10^7$	$1.325X10^7$-$3.856X10^7$	$1.031X10^7$-$3.215X10^7$	$1.021X10^7$-$3.264X10^7$	$1.241X10^6$-$3.341X10^6$
X2	0.133	0.660	1.057	0.124	0.776	1.078	0.898	0.681	0.492	1.005	0.703	1.095
P value	0.936	0.719	0.589	0.940	0.678	0.583	0.638	0.711	0.782	0.605	0.704	0.579
Regression equation	Y=-1.21+9.13E-8*X	Y=-0.89+7.5E-8*X	Y=-0.75+1.03E-8*X	Y=-1.25+7.3E-7*X	Y=-1.02+6.54E-8*X	Y=-0.87+9.52E-8*X	Y=-1.14+7.31E-8*X	Y=-1.03+1.23E-8*X	Y=-0.64+8.747E-7*X	Y=-1.23+1.02E-7*X	Y=-0.99+1.1E-7*X	Y=-0.75+7.921E-8*X

Table 5: LC_{50} and LC_{90} values for *L. giganitum* fungal suspension in adult of *C. molestus* and *A. aegypti*

LC	*A.aegyptia*								*C.molestus*							
	male				Female				male				Female			
	24	48	72	168	24	48	72	168	24	48	72	168	24	48	72	168
LC_{50} value	$1.078x10^7$	$1.008x10^7$	$5.391x10^6$	$4.18x10^5$	$1.109x10^7$	$9.209x10^6$	$5.704x10^6$	$1.055x10^6$	$9.373x10^6$	$7.675x10^6$	$2.394x10^6$	$2.927x10^5$	$9.750x10^6$	$7.432x10^6$	$3.263x10^6$	$4.43x10^5$
Limits 95 %	$8.681x10^6$-$1.271x10^7$	$8.956x10^6$-$1.201x10^7$	$4.151x10^6$-$7.211x10^6$	$2.986x10^6$-$6.782x10^6$	$9.296x10^6$-$1.305x10^7$	$7.802x10^6$-$1.100x10^7$	$4.602x10^6$-$7.304x10^6$	$8.534x10^5$-$1.241x10^6$	$5.283x10^6$-$1.110x10^7$	$4.651x10^6$-$9.625x10^6$	$1.382x10^6$-$4.591x10^6$	$1.825x10^5$-$4.02x10^6$	$5.550x10^6$-$1.165x10^7$	$3.232x10^6$-$9.132x10^6$	$2.561x10^6$-$5.113x10^6$	$1.433x10^2$-$6.782x10^4$

LC	*A.aegyptia*								*C.molestus*							
	male				Female				male				Female			
	24	48	72	168	24	48	72	168	24	48	72	168	24	48	72	168
LC_{90} value	$2.584x10^{7}$	$3.050x10^{7}$	$2.274x10^{7}$	$8.558x10^{6}$	$2.507x10^{8}$	$2.458x10^{7}$	$1.992x10^{7}$	$1.026x10^{7}$	$2.337x10^{7}$	$2.376x10^{7}$	$1.133x10^{7}$	$8.853x10^{6}$	$2.287x10^{7}$	$2.024x10^{7}$	$1.199x10^{7}$	$8.821x10^{6}$
Limits 95 %	$1.861x10^{7}$-$3.682x10^{7}$	$2.200x10^{7}$-$4.542x10^{7}$	$1.864x10^{7}$-$3.844x10^{7}$	$6.503x10^{6}$-$1.135x10^{7}$	$2.106x10^{8}$-$3.706x10^{8}$	$1.658x10^{7}$-$3.556x10^{7}$	$1.791x10^{7}$-$2.595x10^{7}$	$9.226x10^{6}$-$1.722x10^{7}$	$1.236x10^{7}$-$3.754x10^{7}$	$1.486x10^{7}$-$3.952x10^{6}$	$9.311x10^{6}$-$1.551x10^{7}$	$7.254x10^{6}$-$9.903x10^{6}$	$1.689x10^{7}$-$3.059x10^{7}$	$1.210x10^{7}$-$3.015x10^{7}$	$9.532x10^{6}$-$1.356x10^{7}$	$6.811x10^{6}$-$1.115x10^{7}$
X2	0.644	0.520	1.406	1.588	0.726	0.783	0.415	1.458	1.078	0.859	3.979	2.034	1.199	0.499	3.129	2.043
P value	0.725	0.771	0.495	0.452	0.696	0.676	0.813	0.482	0.583	0.651	0.137	0.362	0.549	0.779	0.209	0.360
Regression equation	Y=-0.92+8.75E-8*X	Y=-0.64+6.4E-7*X	Y=-0.4+7.56E-7*X	Y=-0.07+1.57E-7*X	Y=-1.03+9.5E-7*X	Y=-0.77+8.57E-7*X	Y=-0.52+9.09E-7*X	Y=-0.15+1.4E-7*X	Y=-0.87+9.52E-8*X	Y=-0.62+8.15E-8*X	Y=-0.36+1.48E-7*X	Y=-0.04+1.57E-7*X	Y=-0.96+1.03E-7*X	Y=-0.75+1.02E-7*X	Y=-0.49+1.52E-7*X	Y=-0.06+1.3E-7*X

Table 6: LC_{50} and LC_{90} values for *B. bassiana* fungal suspension in adult of *C. molestus* and *A. aegypti*

LC	*A.aegyptia*								*C.molestus*							
	male				Female				male				Female			
	24	48	72	168	24	48	72	168	24	48	72	168	24	48	72	168
LC_{50} value	$1.464x10^{7}$	$1.033x10^{7}$	$4.461x10^{6}$	$2.315x10^{6}$	$2.001x10^{7}$	$1.078x10^{7}$	$9.108x10^{6}$	$3.375x10^{6}$	$1.245x10^{7}$	$9.769x10^{6}$	$4.314x10^{6}$	$1.435x10^{6}$	$1.456x10^{7}$	$1.076x10^{7}$	$6.159x10^{6}$	$2.042x10^{6}$
Limits 95 %	$1.004x10^{7}$-$1.677x10^{7}$	$8.824x10^{6}$-$1.302x10^{7}$	$3.261x10^{6}$-$6.211x10^{6}$	$1.614x10^{6}$-$3.601x10^{6}$	$1.141x10^{7}$-$2.217x10^{7}$	$8.243x10^{6}$-$1.307x10^{7}$	$7.984x10^{6}$-$5.610x10^{6}$	$2.071x10^{6}$-$5.312x10^{6}$	$9.429x10^{6}$-$1.412x10^{7}$	$7.939x10^{6}$-$1.306x10^{7}$	$3.218x10^{6}$-$5.771x10^{6}$	$1.135x10^{6}$-$1.931x10^{6}$	$1.136x10^{7}$-$1.755x10^{7}$	$8.801x10^{6}$-$1.206x10^{7}$	$4.109x10^{6}$-$8.123x10^{6}$	$1.022x10^{6}$-$3.542x10^{6}$
LC_{90} value	$3.256x10^{7}$	$2.637x10^{7}$	$1.500x10^{7}$	$1.319x10^{7}$	$4.367x10^{7}$	$2.584x10^{7}$	$2.677x10^{7}$	$1.405x10^{7}$	$2.874x10^{7}$	$2.667x10^{7}$	$1.365x10^{7}$	$1.123x10^{7}$	$3.237x10^{7}$	$2.752x10^{7}$	$1.908x10^{7}$	$1.089x10^{7}$
Limits 95 %	$2.036x10^{7}$-$4.674x10^{7}$	$1.430x10^{7}$-$3.541x10^{7}$	$1.108x10^{7}$-$1.788x10^{7}$	$1.118x10^{7}$-$1.959x10^{7}$	$3.167x10^{7}$-$5.840x10^{7}$	$1.710x10^{7}$-$3882x10^{7}$	$1.734x10^{7}$-$3.832x10^{7}$	$1.201x10^{7}$-$1.801x10^{7}$	$1.974x10^{7}$-$3.670x10^{7}$	$1.863x10^{7}$-$3.607x10^{7}$	$1.121x10^{7}$-$1.975x10^{7}$	$9.221x10^{6}$-$1.400x10^{7}$	$2.347x10^{7}$-$4.867x10^{7}$	$1.232x10^{7}$-$3.342x10^{7}$	$1.002x10^{7}$-$3.093x10^{7}$	$8.891x10^{6}$-$1.521x10^{7}$
X2	0.321	0.583	0.736	2.396	0.891	0.644	0.499	1.661	0.739	0.536	0.376	1.595	1.110	0.356	0.165	1.036
P value	0.852	0.747	0.692	0.302	0.640	0.725	0.779	0.436	0.691	0.765	0.829	0.451	0.574	0.837	0.921	0.596

LC	*A.aegyptia*								*C.molestus*							
	male				Female				male				Female			
	24	48	72	168	24	48	72	168	24	48	72	168	24	48	72	168
Regression equation	Y=-1.05+7.32E-8*X	Y=-0.83+8.18E-7*X	Y=-0.55+1.23E-7*X	Y=-0.28+1.2E-7*X	Y=-1.1+5.86E-7*X	Y=-0.92+8.75E-7*X	Y=-0.66+7.37E-7*X	Y=-0.41+1.22E-7*X	Y=-0.99+8.2E-8*X	Y=-0.74+7.73E-8*X	Y=-0.59+1.38E-7*X	Y=-0.19+1.32E-7*X	Y=-1.06+7.75E-7*X	Y=-0.83+7.77E-7*X	Y=-0.61+9.97E-7*X	Y=-0.3+1.46E-7*X

Table (5,6) shows the effects of different concentrations of *L. giganteum* and *B. bassiana* fungal suspensions on adult mosquitoes of *A. aegypti* and *C. molestus*. The mortality rates for females of both mosquito species were 65% and 67.5% for 3 $\times 10^5$ spore/ml of *L. giganteum* and 60% and 65% respectively for males and females of both species at the same concentration. The lowest mortality rates were observed at the lower concentration 3 $\times 10^2$ spore/ml, which were 40% and 45% for males and 37.5% and 42.5% for females. For *B. bassiana*, the highest mortality rates were at 2 $\times 10^5$ spore/ml with 52.5% and 57.5% and 47.5% and 55% for males and females of both mosquito species, respectively. The lowest mortality rates at the lower concentration 2×10^2 spore/ml were 30% and 35% for males and 27.5% and 40% for females. No mortality was observed in the control treatment.

Moreover, the table indicates a positive relationship between the concentrations of fungal suspensions and both mortality rates and exposure duration. Mortality rates increased for all adults with longer exposure times. Statistical analysis showed crucial differences in adult mortality based on the concentrations used and also significant differences based on gender, with females showing greater resistance than males. *A. aegypti* were more sensitive for fungal suspensions than to *C. molestus*, as evident from the LC_{50} values (Tables 5 and 6). The lowest LC_{50} values were 4.18×10^5 and 2.927×10^5 spore/ml for males and 1.055×10^6 and 4.43×10^5 spore/ml for females with *L. giganteum*. For *B. bassiana*, LC_{50} values were 2.315×10^6 and 1.435×10^6 spore/ml for males and 3.375×10^6 and 2.042×10^6 spore/ml for females after 168 hours.

The results are similar to [59], who reported that exposure of *Oc. soerrensis* adults to *T. cylindrosporum* fungal suspension at a concentration of 5×10^5 spore/ml resulted in 50% mortality after five days, increasing to 100% after nine days [60], stated that the LT50 value was 1.9 days for *An. stephensi* adults exposed to *M. anisopliae* at 1.6 $\times 10^{10}$ spore/ml [61], found that the LT50 value was 3.5 days for *An. gambiae* adults exposed to *B. bassiana* spores, while it was 3.49 days for the same mosquito species exposed to *M. anisopliae* [62]. The LT50 value was 4.1 days for *Ae. aegypti* females exposed to *M. anisopliae* at 1.6×10^{10} spore/ml [63, 31], found that using *C. keratinophilum* for controlling *Cx. quinquefasciatus* resulted in 90% mortality for males and 86.66% for females after five days.

Regarding other medical insect species [64], found that male tsetse flies *G. morsitans* were more sensitive to *M. anisopliae* and *B. bassiana* than females. This contrasts with [65], who reported that female tsetse flies were more sensitive to *M. anisopliae*, with mortality rates of 98.8% for females and 89.6% for males.

Three concentrations (1×10^8, 1×10^6, 1×10^4)spore/ml of the commercial fungal suspension of *B. bassiana* were tested on different stages of the *T. castaneum* insect. The results showed that the second larval stage was more sensitive to the fungal spores compared to the fifth larval stage and adults. At the concentration of 1×10^8 spore/ml, the mortality rate for the second larval stage was 97.5% after 15 days of treatment. The average number of eggs laid by adults treated with the fungal suspension of *B. bassiana* at 1×10^4 spore/ml was 40.0 eggs/female, compared to 98.1 eggs in the control treatment [66].

The mechanism of action of pathogenic fungi against adults (males and females) is through contact. After spraying the adult with the fungal suspension, the fungus penetrates the insect's body wall and enters the body cavity, where it starts attacking various tissues and disrupting the insect's immune system. The fungus continues to grow and reproduce until the insect's body is filled with hyphal growths. The fungus then sends conidiophores outward, followed by the formation of fruiting bodies, leading to the death of the insect [67].

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