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Research article

Pathogenicity and Efficacy of *Beauveria bassiana* against the Larval and Adult Stages of *Culex pipiens*.

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ABSTRACT

Diseases transmitted by mosquitoes pose a threat to global public health. *Culex pipiens* transmits West Nile virus. The eco-friendly biological control, recruited by *Beauveria bassiana*, is a promising strategy. The current study aims to employ entomopathogenic fungi (*B. bassiana*) against the larval and adult stages of *Cx. pipiens* as an alternative method to chemical pesticides. The study investigated the effects of conidial suspension concentrations (3 x 105, 3 x 106, and 3 x 107 spores/ml) on four larval stages of Cx pipiens. The first instar was the most susceptible and showed significantly higher mortality at higher concentrations. The LC50 values for the first larval instar were 2.935 x 10^6 and 5.241 x 10^5 spores/ml after exposing for 24 and 72 h, respectively. For the second instar, the values were 5.988 x 10^6 and 1.845 x 10^6 spores/ml; for the third instar, 6.696 x 10^6 and 1.868 x 10^6 spores/ml; and for the fourth instar, 8.913 x 10^6 and 4.45 x 10^6 spores/ml, in the same intervals of time. Furthermore, mortality percentages for adults were 1.245 x 10^7 and 4.314 x 10^6 spores/ml for males, and 1.456 x 10^7 and 6.159 x 10^6 spores/ml for females after 24 and 72 h of treatment, respectively. The findings demonstrate that *B. bassiana* exhibits significant pathogenicity against both larval and adult stages of *Cx. pipiens*, with efficacy increasing at higher concentrations and longer exposure times. These results support its role as an eco-friendly biological control agent in reducing mosquito populations and limiting the transmission of West Nile virus.

Keywords: *Beauveria bassiana*, biological control, *Culex pipiens*, Entomopathogenic fungi.

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1. INTRODUCTION

Culex pipiens mosquitoes are known to be significant vectors of a number of medically significant pathogens, such as viruses that cause diseases such as West Nile virus and dengue fever [1]. Moreover, they are also important in the spread of *Wuchereria bancrofti*, the etiological agent of lymphatic filariasis (elephantiasis), a debilitating disease, which is a significant threat to the global public health. Lymphatic filariasis is estimated to afflict over 700 million individuals with around 103 million people at risk of contracting the disease in over 80 countries [2]. Control of vectors is usually considered more practical and efficient than direct control of pathogens; therefore, historically, control of mosquito-borne diseases has focused on reducing mosquito numbers. The most common method of mosquito management has been chemical control which has mostly been carried out using synthetic insecticides and is still in use today. Nevertheless, the widespread application of these chemicals has caused certain serious environmental issues, i.e., air, water and soil pollution, thus destroying the ecological balance and worsening the environmental quality. Moreover, the target mosquito populations have shown an astounding ability to be resilient to these insecticides over time, reducing their effectiveness and constituting a significant challenge to the sustainable approach to the management of vectors [3].

Although different extracts of plants have been used [4] and insect growth regulators (IGRs) have been used, these methods have failed to suppress the mosquito population entirely, in

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large part because the insects have adapted and acquired resistance [6]. As a result, an increasing interest in finding alternative control measures, especially biological based ones, has emerged. Entomopathogenic fungi have been identified as one of these promising biocontrol agents due to their extensive distribution in nature, affordability, and high level of host specificity [7]. Not problematic to use higher concentrations because they are safer for human and surrounding environment [8].

Among them, a particular one is *B. bassiana* because it is one of the most important entomopathogenic organisms that can infect and cause disease to insects [6]. It is distinguished by extensive distribution, easy recognition and capability to create strong spores that are able to withstand harsh environmental factors. Because of these features, it is able to attain epizootic levels though its activity is tightly connected with environmental conditions like humidity and temperature [9].

The goal of this study was to isolate pathogenic fungi from wheat-producing buildings and evaluate their effectiveness against the larvae and adults of *Cx. pipiens* located in several locations. Additionally, this study attempted to determine if *B. bassiana* could be used as a safe and eco-friendly biological control for mosquitoes (a type of vector).

2. MATERIALS AND METHODS

2.1. Fungal Isolation and Cultivation

Soil samples were collected from wheat crops in the Al-Diwaniyah province (Al-Shamiyah region) using standard soil sampling protocols [10]. Each sample was randomly selected, and five sites within the soil profile were chosen. Subsamples were collected from the top 10 cm of the soil to obtain a final product of 200 g. Sterile distilled water was used to prepare three serial dilutions (10^{-1}, 10^{-2}, and 10^{-3}). The 10^{-2} and 10^{-3} dilutions were then inoculated at 0.1 mL onto Petri dishes containing sterile culture media. A sterile Drigalsky spatula was used to evenly spread the dilutions across the surface of the medium.

Two selective media were used: (1) Semi-selective media consisting of glucose (40 g/L), peptone (10 g/L), thiabendazole (0.004 g/L), chloramphenicol (0.5 g/L), crystal violet (0.01 g/L) and agar (15 g/L) in distilled water (pH 6.0); and (2) Oatmeal media consisting of oat flakes (20 g/L), hexadecyltrimethylammonium bromide (CTAB; 0.6 g/L), chloramphenicol (0.5 g/L), and agar (15 g/L) in distilled water (pH 6.0). The plates were incubated at 28 ± 1 °C for 5–14 days and examined daily for fungal growth.

2.2. Morphological Features of the Fungal Isolate

Fungal isolates were characterized morphologically by examining macroscopic characteristics of the colony such as its color (both upper and lower surfaces), type of morphology, texture of surface, margins, and elevation, and by measuring the characteristics of the hyphal and conidiophore structures and the size of the conidia after observing them microscopically [11]. The reproductive structures were observed and measured using an ocular micrometer at both 400× and 1000× magnification and photo-documented using a Carl Zeiss Optical Microscope (Model 467065-9902-18VA)..

2.3. Establishment of Permanent Cultures of *Cx. pipiens*

Larvae at various developmental stages were collected from drainage sites in Al-Diwaniyah province using a long-handled scoop, transported to the laboratory in plastic containers, and reared in dechlorinated water. Larvae were fed a standardized diet consisting of wheat, corn, protein, and rice (1:1:1:0.25 ratio; 2 g per container). Species identification was confirmed at the Natural History Museum, University of Baghdad, using standard taxonomic keys. Fig. 1 shows the sample collection, laboratory cultivation, and morphological features of *Cx. pipiens* larvae.

2.4. Effect on Mortality Percentages of the Four Larval Instars

Each of the four larval instar stages was selected at random, with 40 larvae per concentration of the fungal suspension being tested. Larvae were distributed among four containers, each containing 100 mL of a fungal concentration, and the fourth contained sterile distilled water (control treatment). After exposure, the treated larvae were carefully transferred with a fine brush into 250 mL beakers containing sterile distilled water mixed with 10 mg of larval diet. The beakers were incubated at 28 °C under a 14-hour photoperiod. Mortality percentages were recorded at 24 and 72 hours post-treatment [12].

2.5. Effect on Mortality Percentages of Adult *Cx. pipiens*

A large enough quantity of the pupae was gathered away out of the stock culture and put into 10-ml tubes, one at a time, and then the holes filled with cotton until an adult was developed. Glass beakers 1 liter were arranged, with a small dish with a cotton pad wetted in a 10% sugar solution placed in each. All beakers were sprayed with 5 ml of the corresponding fungal suspension using a manual sprayer at a distance of about 15 cm; the control treatment was sprayed with sterile distilled water. Then ten newly emerged adults (male and female separately) were put into the treated beakers with the help of an aspirator. Each concentration (including the control) was reproduced three times in the experiment. The incubation period was 28 °C and mortality rates were taken after 24 and 72 hours [13].

2.6. Laboratory Pathogenicity Assays of Fungal Species

Pathogenicity tests were conducted to assess the effects of various doses of *B. bassiana* under ambient laboratory conditions (mean temperature 28 °C, relative humidity 53%). In each assay, a random number of insects (15 adults) were placed in sterile plastic containers.

The fungal inoculum was prepared by inoculating 14-day-old cultures grown on Potato Dextrose Agar (PDA) with 5 mL of sterile distilled water in 9-cm Petri dishes. Tween-80 was added as a surfactant. Conidia were harvested using a sterile glass rod, and the filtrate was homogenized on a magnetic stirrer for 10 minutes. The mixture was then filtered through a glass funnel lined with sterile gauze, and 5 mL of distilled water was added to the suspension to maximize conidial recovery. The 10 mL filtrate was transferred to a glass flask, and the resulting suspension was labeled the stock suspension [10].

An aliquot of 1 mL was examined using an improved Neubauer hemocytometer to determine the conidial concentration. The number of conidia in the four corner large squares was counted to obtain the mean number of conidia per square. This average was then multiplied by $1x10^4$ to obtain the number of conidia per milliliter. The following dilution formula was used to obtain the desired experimental concentrations [14]: C1V1 = C2V2. Concentrations were prepared: 3×10^5, 3×10^6, 3×10^7 spore/ml.

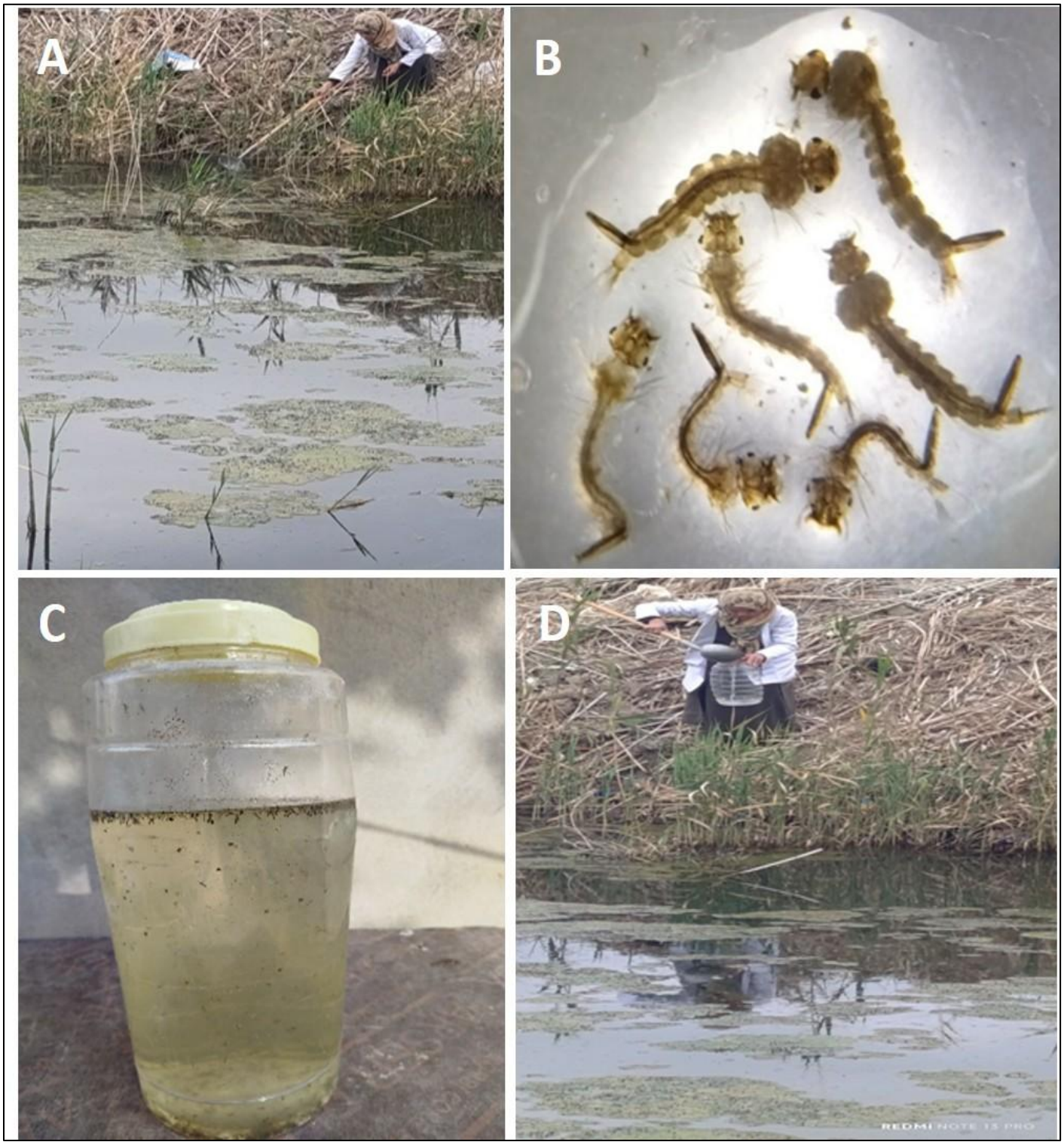

Fig. 1. Images of cultures of *Cx. pipiens*. A and D show the collection of mosquito larvae and pupae (larvae before they become adults, or pupae) from a stagnant water habitat (a natural breeding site) using a standard dipping method (a way to collect a sample without disturbing it or the surrounding area) in the field. B shows the characteristics of 4th (last) instar (young) larvae of Cx pipiens are shown. Cx pipiens young larvae have a special part of their bodies called a "siphon" that allows them to breathe at the water's surface. C shows the laboratory culture vessel containing the collected specimens, which can be observed, raised, and maintained as a colony of Culex pipiens.

2.7. Statistical Analysis

The experiments were conducted following a two- or three-factor model using CRD analysis. The mortality ratios were corrected using Abbott's equation. The corrected percentages were converted to angular values using the Arcsine Transform to ensure normal distribution. Statistical analysis was carried out to assess differences between treatments via LSD analysis at a significance level of probability ≤ 0.05 [15]. Mortality percentages were corrected using [16].

3. RESULTS

3.1. Morphological characterization of *B. bassiana*

Fungal isolates belonging to *B. bassiana* were selected for this study. In macroscopic examination, the colonies exhibited a white to creamy, powdery appearance with irregular margins (Figure 1). Microscopic examination revealed well-defined reproductive structures and conidia with typical morphology, size, and pigmentation. Septate hyphae and conidiogenous cells, measuring 5.4-8.7 µm in length and 2.0-2.8 µm in width (SD: 0.6-1.0 and 0.1-0.7 µm, respectively), were used to characterize the isolates. These cells had a wide basal area and a small apical extension (rachis), on which many conidia were produced in a typical sympodial pattern. The conidia were hyaline, nonporous, and spherical or sub-spherical, with a mean diameter of 1.7-2.3 µm (SD: 0.5-0.6 µm).

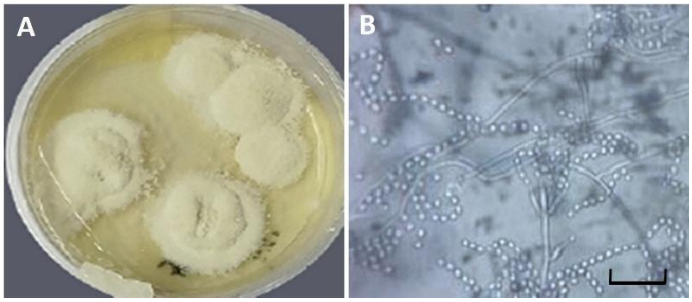

Fig 1. Morphological characteristics of the isolated entomopathogenic fungus. **(A)** Fungal colonies grown on SDA medium showing typical white, cottony, circular growth of *Beauveria bassiana*. **(B)** Microscopic structure of *B. bassiana* stained with Lactophenol Cotton Blue (LPCB), showing hyphal network and characteristic conidial arrangement (scale bar: ????? µm).

3.2. Larval Stage Susceptibility to *B. bassiana*

Table 1 illustrates the effect of different *B. bassiana* conidial concentrations on the mortality of *Cx. pipiens* larval instars. The mortality percentage for the first instar was 5.241x 10^5 and 2.935 x 10^6 spores/ml after 24 and 72 hours, respectively. For the second instar, values were (1.845 x10^6 and 5.988 x10^6) spores/ml for the third, (1.868 x 10^6 and 6.696 x 10^6) spores/ml and for the fourth, (4.45 x10^6 and 8.913 x10^6) spores/ml at the same intervals. No mortality was observed in the control group, indicating a direct correlation between concentration and mortality rates. Furthermore, a positive relationship was evident between exposure duration and mortality across all four instars.

3.3. Adult Susceptibility and Sex-Specific Responses

Table (2) shows the impact of *B. bassiana* on adult *Cx. pipiens*, the mortality percentage for males were (1.245x 10^7 and 4.314 x 10^6) spores/ml while for females, they were (1.456 x10^7 and 6.159 x10^6) spores/ml after 24 and 72 hours, respectively. The data supports the fact that there is a direct correlation between the conidial concentration, exposure time, and mortality rates. Statistical comparison showed that there were significant differences in percent adult mortality according to concentration and sex with males having higher resistance compared to females, this is because the high concentration results in higher mortality since there are more germinating spores that attack the host hence affecting the immune system of the insect.

4. DISCUSSION

Insects can have varying levels of effective immune defenses against fungal pathogens and thus will have different levels of susceptibility to those pathogens; but the variability of these defenses is not distributed consistently among insect populations. The immune system can develop sufficient immune defenses at low conidial loads; however, there is a significant reduction in the immune system's ability to protect against pathogenic fungi at high conidial loads. The sexual dimorphic nature of insect immune systems considerably complicates our understanding of the role of fungal biocontrol agents [17, 18].

Male and female *Cx pipiens* use very different immunological strategies that are representative of their unique ecological roles and physiological limitations [18]. Male mosquitoes employ what would be considered a constitutive immune strategy that has a high level of basal expression of immune-related genes (i.e., sustained immune defenses) even when not exposed to pathogens. This constant state of immunological readiness allows male mosquitoes to have a highly rapid and complete immunological response (immune defense) to fungal pathogens upon exposure. This constant state of protection is due, in large part, to the fact that the male mosquito's diet consists exclusively of plant sugars and floral nectar, which provide the nutritional requirements for sustaining the functioning of the immune system. On the other hand, female *Cx pipiens* have developed a tolerance-based immunological strategy. Following blood meals, they experience profound physiological and hormonal fluctuations that substantially modulate their immune competency [19]. Rather than attempting to clear pathogens completely—an energetically expensive proposition—females maintain a moderate immune response that permits coexistence with certain fungal loads.

Table 1. Lethal concentration (LC_{50} and LC_{90}) values of *B. bassiana* suspension against different larval instars of *Cx pipiens* at 24 and 72 h, including 95% confidence limits, chi-square (χ^2), *P*-values, and regression equations.

	Cx. pipiens Larval instar							
	1 instar		2 instars		3 instars		4 instars	
LC	24	72	24	72	24	72	24	72
LC_{50}value	2.935X10^6	5.241X10^5	5.988X10^6	1.845X10^6	6.696X10^6	1.868X10^6	8.913X10^6	4.45X10^6
Limits 95%	1.544X10^6-5.856X10^5	2.457X10^6-.10^6X10^6	3.722X10^6-9.233X10^6	9.112X10^6-2.131X10^6	5.123X106-7.692X10^6	1.132X10^6-2.733X10^6	7.223X10^6-9.824X10^7	3.122X10^6
LC_{90} value	1.084X10^7	8.540X10^6	1.849X10^7	1.248X10^7	1.916X10^7	1.235X10^7	2.179X10^7	2.094X10^7
Limits 95%	5.871X10^6-2.785X10^7	4.598X10^6-6.335X10^7	1.298X10^6-2.143X10^7	9.723X10^6-1.672X10^7	1.122X10^7-2.732X10^7	9.224X10^6-2.162X10^7	1.621X10^6-3.251X10^7	1.322X10^7 - 3.314X10^7
χ^2	0.633	0.537	0.818	0.613	0.176	0.651	0.627	0.411
P value	0.729	0.764	0.664	0.736	0.916	0.722	0.731	0.814
Regression equation	Y = -0.48+1.63E-8*X	Y=-0.08+1.6E-7*X	Y=-0.62+1.04E-8*X	Y=-0.14+1.14E-7*X	Y=-0.69+1.03E-8*X	Y=-0.23+1.23E-7*X	Y=-0.89+1.12E-7*X	Y=-0.35+7.85E-7*X

Table 2. Lethal concentration (LC_{50} and LC_{90}) values of *B. bassiana* fungal suspension against adult male and female *Cx pipiens* at 24 and 72 h, including 95% confidence limits, chi-square (χ^2), P-values, and regression equations.

	Male *Cx. pipiens*		Female *Cx. pipiens*	
LC	24	72	24	72
LC_{50} value	$1.245x10^7$	$4.314x10^6$	$1.456x10^7$	$6.159x10^6$
Limits95%	$9.429x10^6$-$1.412x10^7$	$3.218x10^6$-$5.771x10^6$	$1.136x10^7$-$1.755x10^7$	$4.109x10^6$-$8.123x10^6$
LC_{90} value	$2.874x10^7$	$1.365x10^7$	$3.237x10^7$	$1.908x10^7$
Limits95%	$1.974x10^7$-$3.670x10^7$	$1.121x10^7$-$1.975x10^7$	$2.347x10^7$-$4.867x10^7$	$1.002x10^7$-$3.093x10^7$
χ^2	0.739	0.376	1.110	0.165
P value	0.691	0.829	0.574	0.921
Regression equation	Y = -0.99+8.2E-8*X	Y = -0.59+1.38E-7*X	Y=-1.06+7.75E-7*X	Y=-0.61+9.97E-7*X

This evolutionary trade-off prioritizes reproductive success, allowing females to conserve energetic resources for the demanding processes of oogenesis and oviposition rather than committing them to futile pathogen elimination efforts.

An understanding of the mechanistic basis of fungal pathogenesis is fundamental to understanding the promise of entomopathogenic fungi as biological control agents. The infection process occurs through direct contact in both adult males and females [20]. Fungal spores are applied to an insect's surface initiating an invasive cascade. The fungus penetrates the insect's protective cuticle to systematically colonize different tissues in the hemocoel while evading host immune responses. Entomopathogenic fungi have multiple routes for invasion, including directly breaching the cuticle, accessing the digestive tract, penetrating through the respiratory spiracles, or through existing wounds [21]. The fungus uses the secretion of powerful degrading enzymes (proteases, lipases, and chitinases) to dismantle the structural components of the insect exoskeleton allowing it to penetrate [21, 22]. After the successful colonisation of the host, fungal hyphae spread throughout the host and essentially consume the entire insect host. Finally, the production of conidiophores and fruiting body marks the completion of the infection process and leads to the insect's death. In addition, some entomopathogenic fungi are able to persist in the environment when there is no host. Thus, the potential for their impact extends beyond direct contact situations. [22].

In the world of fungi used as biological control agents, *B. bassiana* has become one of the most successful. It has a very broad host range and shows significant effectiveness in controlling insects. Studies show that this type of fungus can infect approximately 200 different types of insects, with about half of them being from two specific groups (Lepidoptera and Coleoptera) [23]. Like many other pathogenic fungi, *B. bassiana* infects via contact through the growth of conidia on the cuticle of an insect. When *B. bassiana's* conidia land on a suitable insect, and the environment is conducive to germination, these conidia will begin developing into new fungal mycelium by extending their germ tube from the conidia. This will occur at the site where the conidia are in contact with the insect's cuticle. Through the extension of the germ tube, *B. bassiana* will secrete specific degradative enzymes that will degrade the cuticle components of the insect, starting with the protein, then chitin, and finally lipids. The unique appeal of *B. bassiana* for use in integrated pest management systems is that it is selective for host insects while posing little risk to non-targets. Additionally, *B. bassiana* can be produced in large quantities at a low cost and relatively easy to prepare for commercial application [24].

Numerous studies have thoroughly documented the effectiveness of *B. bassiana* in combatting a wide variety of insect pests. The fungus produced 58-91% mortality rates of Aphis craccivora (cowpea aphid) seven days post treatment [25] due to the secretion of fungal chitinase/lipase enzymes and a variety of toxins that permit the fungus to penetrate deeply into the insect's body [26]. One of the most noteworthy indications of *B. bassiana*'s effectiveness as a disease vector pathogen is in mosquito populations, especially Anopheles gambiae. A study on the effect of *B. bassiana* on adult Anopheles gambiae *Cx pipiens* determined a LT_{50} (median lethal time) of 3.5 days, while another study on the effect of Metarhizium anisopliae had a similar LT_{50} of 3.49 days [20]. Larval *Cx pipiens* also appear to be quite susceptible; ingestion of fungal conidia by larvae leads to toxic metabolite production that causes septicemia and death [27, 28]. Due to its effectiveness against both adult and larval forms, *B. bassiana* presents significant promise as a tool for vector control.

The broad-spectrum potential of *B. bassiana* is further evidenced by its activity against numerous economically important pest species. In studies examining Aphis fabae (black bean aphid), a 100% culture filtrate concentration of *B. bassiana* produced mortality rates of 52.17% in nymphs and 54.10% in adults [18], indicating its utility as a versatile biological control agent. Against Spodoptera frugiperda (fall armyworm), *B. bassiana* achieved substantial mortality rates, though specific efficacy varied depending on multiple biological and environmental factors. These variations in mortality rates across different target insects may be attributed to differences in spore germination rates, growth factor availability, enzyme production efficiency, spore adhesion strength, and various other circumstances that collectively support fungal survival and proliferation [29]. This variability underscores the importance of optimizing application conditions and spore concentrations for specific target pests.

B. bassiana (Beauveria bassiana) showed concentration-dependent efficacy in laboratory studies. Research on Tribolium castaneum identified a positive correlation between mortality rates and spore concentrations, the highest concentration tested of $17x10^8$ spores/ml provided 93.33% mortality of adults by 10 days post-treatment [30]. This relationship illustrates the importance of spore concentration for successful infection, corresponding to previous findings on the performance of the insect immune system at different conidial loads. By integrating knowledge of sexual dimorphism in insect immune systems with evidence of *B. bassiana* efficacy, researchers can develop improved biocontrol options. Given the unique immune responses of male and female insects, researchers may develop more targeted applications based on sex-related vulnerability differences. Combined with *B. bassiana*'s selectivity towards specific insect hosts, low cost, broad-spectrum activity, and substantial environmental persistence, researchers can increasingly rely on this fungus as part of a sustainable, integrated pest management program. Further research on interactions between host immune mechanisms and virulence factors will enable greater use of entomopathogenic fungi as precision biological control agents against agricultural pests and disease vectors.

5. CONCLUSION

Findings from this investigation have provided much insight into the association of entomopathogenic fungi with mosquito larvae within Al-Diwaniyah City. Specifically, the study concluded that *B. bassiana* is a very effective means of biologically controlling *Cx. pipiens*, particularly within its early larval stages.

As such, *B. bassiana* serves as an environment-friendly option to chemical insecticides and demonstrates that conidial concentration and fecundity are related to the level of mortality in treated mosquitoes. The results of this research also indicate varying degrees of susceptibility among different instar stages of larval mosquitoes as well as death via mechanical and enzymatic mechanisms when present on these species. Therefore, in order to successfully control *Cx. pipiens*, the use of *B. bassiana* provides a much more environmentally safe alternative than chemical pesticides and could improve sustainable agriculture for wheat production by providing an environmentally friendly alternative for controlling mosquito populations and making possible the use of only the most virulent strains of *B. bassiana* for large-scale implementations.

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Conflict of interest

The author declares that she has no conflict of interest.

Ethical Approval

This project was approved by the ethics committee at the University of Al-Qadisiya (No. 2778; 12/3/2025).

CRediT authorship contribution statement

Abidfalhy MM: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Validation, Resources, Project administration, Writing of the original draft, and Writing – review and editing.

The author has read and agreed to the published version.

Availability of data and materials

All information used is available in the cited literature and in request.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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