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Effect entomopathogenic fungi in control *Culex pipiens*

Mohammed Qasim Waheeb
Dept. of Biology/College of Science/Al Muthanna University
Samawah,66001, Iraq
E-mail: mhmdkas@mu.edu.iq

2nd Sadik Thajeb Ali
Dept. of Biology/ College of Science/University of Thi-Qar
Thi-Qar,64001, Iraq

3rd Haider Radhi Malih
Dept. of Biology/ College of Science/University of Thi-Qar
Thi-Qar,64001, Iraq

***Abstract*— *Culex pipiens* mosquitos are thought to be vectors for many arboviruses, including West Nile virus and encephalitis virus, which have a global impact on human health. The natural management of this pest's aquatic stages is critical for sustaining an insecticide-free environment. The current study focused on the biological and biochemical effects of the entomopathogenic fungi *Beauveria bassiana* and *Trichoderma harizanium* on *Culex pipiens* laboratory colony 3rd instar larvae. The results showed that B. *bassiana* had the highest larval mortality (22%) with a concentration $1x10^6$ spore/ml and the period exposure recorded (36.6%) shortest lethal time (120 hrs), followed by *T. harizanium* (17.3%) with a concentration $1x10^7$ spore/ml and the period exposure recorded (29.1%) shortest lethal time (120 hrs), and had the lowest percent mortality (16%) with the concentration $1x10^3$ spore/ml and period exposure with (6%) longest (24 hrs) for B. *bassiana* and had the lowest percent mortality (4%) with the concentration $1x10^3$ spore/ml for *T. harizanium* and period exposure with 24 hrs (12.5%).**

Keywords— *Culex pipiens, Beauveruia bassiana, Trichoderma harizanium*, entomopathogenic fungi.

I. Introduction

Mosquitoes are dipteran insects that serve as biological and mechanical vectors for a variety of parasites and pathogens that cause communicable diseases. They can transmit enzootic or even epizootic diseases like malaria, dengue fever, and filariasis, among others. The World Health Organization (WHO) created a Global Vector Control Response (GVCR, 2017-2030) to implement long-term vector control strategies (1). Despite its effectiveness, chemical control of vector insects poses a health, environmental, and climatic risk. The relevant situation of insecticide resistance and unsustainable interventions pose challenges to achieving sustainable development goals. The Culicidae are almost all bloodsuckers and are responsible for the spread of many serious diseases (2). The *Cx. pipiens* arouses the most interest due to its widespread distribution in tropical and subtropical countries, which has a socioeconomic impact. To counteract adult resurgence during adult control, vector control strategies typically target the aquatic stages of mosquitoes in their breeding habitat. Chemical larvicides that target mosquito breeding sites cause resistance in the targeted. species as well as long-term secondary effects on non-targeted organisms, harming aquatic fauna (3). Exploring eco-friendly and biological control methods is necessary for developing an alternative strategy for larval control. Entomopathogenic microorganisms hold a significant place among alternative methods of combating insect pests. The use of entomopathogenic fungi to control insect pests yielded promising results, with *Beauveria bassiana*(4)and *Metarhizium anisopliae* (5) being self-sustaining and efficient alternatives for controlling *Cx. pipiens* (6). Both species have a diverse range of isolates that differ in host specificity and origin. By controlling the aquatic stages of mosquitoes, these fungi will help to maintain the ecological balance in the surrounding aquatic habitats. (7). The current study examines the effectiveness of two Entomopathogenic microorganisms against *Cx. pipiens* larvae.

II. Methods

A. Insect Colony Maintenance

A laboratory strain of *Cx. Pipiens* were obtained from the laboratory research insect of the Department of Biology, College of Science University of Thi-Qar, Iraq. The colony was kept in a walk-in chamber insectary at 272 degrees Celsius, 70% relative humidity, and a photoperiod of 12:12 hours. Mosquito larvae were grown in white enamel dishes with 1500 ml of distilled water. The newly hatched larvae were fed fish food (Tetra-Min, Germany). The adult was raised in wooden cages (24 x 24 x 24 cm) with 10% sucrose solution and a pigeon for female feeding.

B. Fungus Culture

Beauveria bassiana (Balsamo), and *Trichoderma harizanium*solates were obtained from the Mycology Center, Ministry of Science, and Technology, Iraq. The isolates were cultured in flasks on Sabouraud dextrose yeast agar (SDYA) medium (8), which contained 40 g glucose, 20 g peptone, 20 g agar, and 2 g yeast extract dissolved in 1000 ml of distilled water. The flasks were autoclaved for 15-20 minutes at 121°C. The media was poured into Petri dishes and ready for inoculation (9).

C. Inoculum Preparations

Fungal cultures were plated into prepared Petri dishes and incubated in darkness at 252 °C for 14 days. Conidial suspensions were made in a laminar airflow chamber by scraping cultures with a sterile inoculation needle and transferring them to 10 ml of distilled water containing

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0.05% Tween 80. For 10 minutes, the mixture was stirred. The hyphal bodies were removed from the mixture by filtering it through a fine mesh sieve. The final suspension's conidial concentration was determined by direct count with a hemocytometer (9, 10).

D. Bioassay

The purpose of the virulence test was to compare the efficacy of the two fungal isolates against Cx. pipiens 3rd larval instars The fungal spore suspension was serially diluted in distilled water containing Tween-80 (0.1%) and stored at 5 oC until use (10). Conidia from the isolates were tested against larvae by adding the fungal suspensions to plastic cups containing 50 ml of distilled water and 25 3rd instar larvae. Each cup was inoculated with 1ml of fungal suspensions containing 1x106, 1x107, and 1x108 spores per milliliter. Control treatments included the addition of distilled water containing Tween-80 (0.1%) (11). Larvae were fed fish food and monitored daily. Mortality was measured at 24, 48, and 72 hours after larval feeding (12).
The following biological parameters were studied to determine the effect of fungal treatment on the mosquito life cycle and female fecundity parameters: mean larval and pupal duration and percentage of pupation. Pupae were sexed and placed in glass globes in pairs. The percentages of adult emergence, adult longevity, female fecundity, and egg fertility were calculated (13).

E. Statistical analysis

One way ANOVA at $p \leq 0.05$ was used to analyse the triplicate data means followed by a Multiple Comparison Test (MCT) to indicate the signfcant differences between means using a statistical analysis system (SAS, 2003).

III. RESULTS

A. Effect suspension to the Beauveria bassaina and Trichoderma harizanium with death mortality on third larvae Culex pipiens

The figures (1-2) have been showing the effect of interface different concentrations from suspensions of Beauveria bassaina and *Trichoderma harizanium* fungi in death rate mortality on the fourth larvae stages for *Culex pipiens*, where exceeding the suspended *B. bassaina* fungus on the suspended *T. harizanium* fungus when used the most concentration $1x10^6$ spores/ml reached death mortality in the third stage of larvae *C. pipiens*, the highest concentration was registered mortality for both fungi at 22% and 17.3% respectively, while the period exposure for the same stage of larvae was mortality for the most period exposure as follow 36.6% and 29.1% respectively.

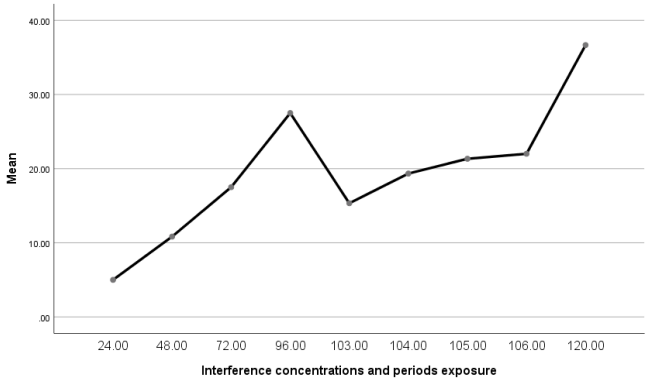

Figure (1) Effect suspension of the *B. bassaina* on larva stage (3) *Cx. pipiens.*

LSD 0.05(con.) =9.5, LSD 0.05 (p.ex.) =5.05, LSD 0.05 (interference)=7.76

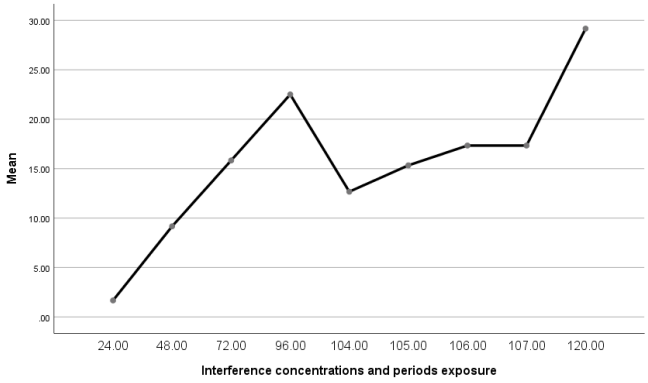

Figure (2) Effect suspension of the *T. harizanium* on larva stage (3) *Cx. pipiens.*

LSD 0.05(con.) =7.83, LSD 0.05(p.ex.) =3.59, LSD 0.05 (interference.)=6.254.

IV. DISCUSSION

The increasing in death rate with increase the concentration attributed to increased number of spores and then increased rate spores growth when attacked the host and impairment the immune system for on the confront for the insect, however the immune system for larvae can defend about body it, if the low concentrations, while the high concentrations may be loss the immune system effective it (14), and the statistical analysis has been proven presence significant differences between the percentage of the larvae stages and it has been proven between the species fungi, while the relationship between the death rate and the age of larvae stages reverse, the reason may be to the immune system is not completely, and as the body system is delicate and which allow penetration easily by the fungus spores (15), the reason different may be to the fungi disparity in death rate to ability these fungi secrete analytic enzymes and toxins effect on action lead to death it. (16), found the exposure of larvae of *Cx.quinquefasciatus* mosquitoes to *B.bassiana* fungus spores led to dead all it with concentration 1x108 spores/ml and the death rate the percentage 97% with a concentration $1x10^7$ spores /ml showed a percentage of death rate *Cx.quinquefasciatus* mosquitoes increased with advance the suspension concentrations *Chrysosporium keratinophilum*, and not different about) the results that previously it was the percentage first stage of larvae for

Cx.quinquefasciatus as follows (77.7, 60, 59.5) % with concentrations($2x10^4$, $2x10^3$, $2x10^2$.) spores/ml these result agreed with the results (17) when describing the relationship between the stages of larvae and for *Aedes aegypti* and death rate, where demonstrated death rate decrease with advancing the age and the death rate for the first and second stage reached 100% follow the third and second stage reached mortality 40% when the treatment with the suspension *Leptolegnia chapmani* with the concentration 3.65x105 spores/ml. The (18) used treatment *An. stephensi* and An. gambiae with suspension M. anisopliae were the percentage mortality to the third and fourth stages less than the percentage mortality to the first and second stages for both species mosquitoes. The (19) showed to exposure the second stage of *Cx. quinquefasciatus* mosquito to the A. niger spores fungus with the concentration 2x106 spores/ml led to death rate with 68.4% after two days from treatment, the injured insect by fungi may be lived the period progression 3-5 days because the grown spores and penetration the mycelium through the breathing tubes that caused suffocation the larvae because the closed breathing tubes, as well as the growth fungus in mid canal of trachea for larvae and drain the foods caused after 72 hours crash the lipid tissues and therefore may be percentage mortality to 100% after 96 hours, and also of some larvae dead during the metamorphosis (20). The (21) added the swallowing of larvae following secretion toxins from the entomofungal pathogen and thus leading toxemia(22).

V. Conclusions

Entomopathogenic fungi are thought to be a naturally occurring microbial control agent against many insects, helping to reduce host population in epizootics. The majority of them begin the infection process in the gut. Fungal enzymes aid in cuticle penetration, and toxin production induces the host's immune response, including activation/deactivation of host enzymes. The fungal propagation within the host body causes a significant depletion of host biomolecule availability, affecting variable parameters in the host life cycle, and eventually host death in a susceptible host. The difference in cuticular structure and chemical composition of the epicuticle influences the host-fungus relationship. Toxicity differences between selected fungal isolates.

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