

The Cumulative Effects of native isolate of Entomopathogenic fungus *Beauveria bassiana* on the developmental stages of *Culex mosquitoes*

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Abstract—This study was conducted in city of Zakho to assess the biological compoundable of effects for entomopathogenic fungus *Beauveria bassiana* on the mosquito (*Culex pipiens* Linnaeus) employing a different concentration (1 x 10³, 2 x 10³, 3 x 10³, 4 x 10³, 5 x 10³ & 6 x 10³ spore) for all juvenile stages of mosquito. The compoundable effect of the fungal isolate affected the hatchability of eggs significantly; rate of death and elongation of the duration for the larvae and pupae of *C. pipiens*, where eggs, larval & pupal mortality were concentration dependent. The high concentration (6 x 10³ spores) of fungal isolate had significant effects on hatchability of the eggs, larval mortality and pupal mortalities (70.37%), (100%) & (97.5%) respectively. The emergence of adults and the most biological activities of the emerging females including fertility were evidently affected. The adult emergence showed highest rate at the concentration 4 x 10³ spores which was 17.54% and the lowest rate of emergence was recorded at the concentration 5 x 10³ spores which was 2.5%. The female's preoviposition period which they were developed from the eggs treated with the fungal isolate, was significantly affected at a concentration of 4000 spores reaching the 7th days. Female fecundity was significantly decreased with increasing concentration of the fungus, and it was found that, at the concentrations of 4000 spores *B. bassiana* the females deposited 42 eggs, and the hatching percent of the eggs at the same concentration (4000 spores) was 76.38%.

Index Terms— *Beauveria bassiana*, *Culex pipiens*, Entomopathogenic fungus, Mosquito, native isolates.

I. INTRODUCTION

The wide use of chemicals (insecticides), causing many problems, like the appearance of the secondary pests which can resist those insecticides, their effects on the wild life, and the contamination of environment with toxic substance [1]. Now a day there are several species of mosquitoes which are responsible for the spreading and transporting many causative agents of diseases such as malaria, Filariasis and others which can resist many insecticides [2 and 3]. For this reason, several researchers started working to find new compounds that can be effective, safe and cannot be dangerous to human health and environmental contamination. From those compounds are the natural extracts of the plants, some insect growth regulators (IGRs) and their analogous like juvenile hormones (JH) [4 and 5]. And also, they can be used as a replacement for conventional synthetic insecticides [6]. Also, the studies were conducted

toward the substitutional methodology (as usage of microorganisms like fungi or bacteria) for utilizing them in the biological control [7 and 8]. Also, the fungi (entomopathogenic) were used as alternative to chemical insecticides for controlling insects [9 & 10]. A widely distributed natural pathogen *Beauveria bassiana* is an important entomopathogenic hyphomycetes which has been used to control a wide variety of harmful insects; like grasshoppers, thrips whiteflies, aphids and many other noxious insects as mosquitoes [11, 12 and 13]. This study was conducted to determine a cumulative effects of entomopathogenic fungi *Beauveria bassiana* on all immature stages of *Culex pipiens* (eggs, larvae & pupae) and the biological activities of adult mosquitoes in Kurdistan Region of Iraq.

II. MATERIALS AND METHODS

This study was conducted in the University of Zakho, Faculty of Science /Department of Biological laboratory during 2017. The colonies of mosquitoes (*Culex*); this culture was maintained under the condition of insectary for two generations which performed by using plastic containers (400 ml) containing tap water, and provided with rabbit chew as food source. After the emergence of adults, they were feeding by 10% sugar solution (using a piece of cotton soaked in it), then these females used for egg deposition [14]. The mosquito species which had been used in this study was classified as *Culex pipiens* L. (Diptera: Culicidae). The conditions of rearing (temperature & relative humidity were 28±1°C & 60-75% respectively) also the conditions includes 12 hrs. photoperiod followed by 12 hours of darkness [15]. The *Beauveria bassiana* which had been collected from dead insects (beetles) in the village Sarceng were washed with 1% sodium hypochlorite solution then after growing, the process of isolation was done on the media (agar); purifying and keeping in freezer until user time. The isolated fungus was prepared according to method described by Abdulla [16]; (for obtaining a stock solution; one gram of fungal spore was mixing with 50 ml of distilled water). Concentrations of 1 x 10³, 2 x 10³, 3 x 10³, 4 x 10³, 5 x 10³ and 6 x 10³ spores were prepared from stock solution. After laying of eggs (deposition), the egg rafts were collected and then transferred to plastic bowls of capacity (250 ml), for each concentration of the entomopathogenic fungus, 4 replicates were used. The biological aspects which had been used in this study were: the hatchability of eggs, growth period of immature stages, mortality (death) rate, and female fertility. All mortality rates were corrected according to Abbott formula [17].

All the data have been analyzed by using the program Graph pad prism 5 (USA), one-way analyses (ANOVA).

III. RESULTS and DISCUSSION

The death rate of *Culex* eggs was affected significantly by the *Beauveria bassiana* isolate at all concentrations used and the mortality of the eggs was concentration dependent ranging between 5.6% – 29.63% at concentrations of 1 x 10³ - 6 x 10³

spores per 250 ml respectively (Table 1).

Table (1): The effect of *Beauveria bassiana* isolate on the rates of mortality for different immature stages of *Culex pipiens*

Concentrations mg/ml	% rate egg death	% rate larval mortality	% rate pupal mortality
0 (control)	2.50	7.60	8.33
1 x 10 ³	5.60	43.75* **	26.34***
2 x 10 ³	10.7 7	51.25*	44.93***
3 x 10 ³	14.7 1	75.66* **	57.30***
4 x 10 ³	18.5 8**	85.70* *	78.30***
5 x 10 ³	23.4 5**	94.5** *	97.5***
6 x 10 ³	29. 63***	100** *	***

The rate of egg's death may be due to the solidifying of egg shell that leads to the death of embryos which make the eggs unable to hatch, or may be due to the fact that the fungus prevents the exchange of gas [18 and 19]. Figure (1) showed the hatched and unhatched raft of eggs which was treated with concentration of 6 x 10³ spore of *B. bassiana*. The mortality rate of larvae was also strongly affected, and all the larvae had been died at a concentration of 6 x 10³ spores, while the concentration of 1 x 10³ spores cause a lowest percentage of death 43.75% (Table 1). When Bukhari et al. [20] treated the larvae of mosquito *An. gambiae* with Shell Sol T-formulated *B. bassiana* spores, they found that the larval mortality rate is concentration dependent when they used low dose (10 mg) giving 61% for larval mortality and 50% with high dose (20 mg) lower than that in the corresponding unformulated treatments. The results of present study showed that when the larvae exposed for 72 hours to the entomopathogenic fungus led to decomposition of the larval body of the mosquitoes (Figure 2), the reason of this result may be attributed to the life cycle of fungus, where after it's penetration and growing on the body surface to complete their life cycle, it consuming the host body [21]. For the pupae, those whose can developed from the treated larvae were also affected by fungal isolate showing a concentration dependent mortality. Since the 100 percent of larval mortality rate was obtained at 6 x 10³ spores' concentration, therefore, there was no pupae appeared at this concentration. The lowest percentage 26.34% of pupal mortality was found at a concentration of 1x10³ while the

concentration of 5 x 10³ spores showed a highest percentage 97.5 % (Table 1). After the infection, some of the larvae and pupae obtained an abnormal shape and their death may reflect for growth inhibitory effect of the fungus (Fig. 3 & 4).

Figure 1: The hatched eggs raft (right side) which exposed to concentration of 6 x 10³ spore of *Beauveria bassiana* and the



left eggs raft is the normal untreated one (20X)



Figure 2: Abnormal fourth instar larva of *Culex pipiens* (resulted from eggs treated with 6 x 10³ spores of *Beauveria bassiana* after 72 hours exposure (28x).



Figure 3: The right side shows the normal untreated larva comparing with the left side of decaying dead larva treated with 6 x 10³ spores of *Beauveria* (28x)



Figure 4: The dead intermediate stage (larval-pupal during the process of molting) resulted from treated egg with 5×10^3 spores of *Beauveria bassiana* (28 x)

Because the failing of adults to emerge, most of them were dead (fig.5). The results of present study illustrated that all the mortalities were dosage-dependent and the adult emergence rate was decreased when the concentrations of the fungal isolate is increasing. The lowest rate of the adult emergence 2.5 % was with concentration of 5×10^3 spores,



Figure 5: Failing of adult's mosquito to emerge (Partially emerged) resulted from egg treated with 4×10^3 spores of *Beauveria bassiana* isolate (28 x).

while the highest rate 73.66 % was at the concentration of 1×10^3 spores (Table 2). When [22] treated the *Culex pipiens* with conidia of *Beauveria bassiana*, they recorded a detracting of 69% among pupae after two weeks exposure, causing a percentage of 31% in adult emergence.

A different results of pupal mortality percentage (85.9%) were obtained by [23] when *An. stephensi* mosquitoes were infected by *Beauveria bassiana* and the adult emergence was 14.1%, similar results have been reported by [24] when they treated *Ceratitis capitata* pupae with entomopathogenic fungi obtaining mortality of 94%. [25] used the exposed eggs of *Haematobia irritans* to the concentration of 106 spores entomopathogenic fungus *B. bassiana*, causing a reduction in adult emergence by 43.8%.

On the other hand, the duration of immature stages of *Culex pipiens*, was affected also by *B. bassiana* which had been illustrated in table (3), and according to its data, the time periods of all immature stages were increased with increasing concentration. The of the mosquitoes was also affected by the fungal isolate in all concentrations especially at concentration $2 \times 10^3 - 5 \times 10^3$ spores where it ranged between 14.5 and 19.5 days respectively. The reason for elongation of developmental period of immature stages of the insect may be due to the inhibition in the larval development which ultimately leads to elongate the larval and pupal stages of mosquito. [21] tested the effects of *B. bassiana* on the *Lutzomyia longipalpis* and they found that the fungus showed increasing of duration in the immature stages.

Table (2): The effect of *Beauveria bassiana* isolate on the adult's emergence & emergence inhibition developed from treated eggs of *Culex pipiens*.

Concentrations mg/ml	% adult emergency	% emergence inhibition
0	91.67	8.33
1×10^3	73.66***	26.34***
2×10^3	55***	45***
3×10^3	42.70***	57.30***
4×10^3	21.70***	78.30***
5×10^3	2.5***	97.50***

Table (3): The effect of *Beauveria bassiana* isolate on the developmental period of different immature stage of *Culex pipiens*.

Concentration mg/ml	Mean Larval duration (days)	Mean Pupal duration (days)	Total duration (days)
0	8	3.5	11.5
1×10^3	8.5	4	12.5
2×10^3	9.25**	5.25**	14.5
3×10^3	10***	6***	16*
4×10^3	10.5***	7.5***	18***
5×10^3	11.25***	8.25***	19.5
6×10^3	11.75***	***	11.75

IV. CONCLUSION

- 1-The fungal isolate of *Beauveria bassiana* was effective against all immature stages of *Culex pipiens* mosquitoes in cumulative effects.
- 2- The cumulative effect of fungal isolate caused the prolonged growth period of immature stages which reached 19.5 days from egg to adult at the concentration 5×10^3 spores.
- 3- It appears that the fertility of the mosquito's females was affected by fungal isolate and the failure of adult's emergence.

Only a few numbers of adults which had been emerged from the treatment with concentrations of 6×10^3 spores, and all of them died before having a chance for reproduction (Table 4). However, the females which developed from larvae treated with concentration of 1×10^3 - 4×10^3 spores of *Beauveria bassiana* deposited a few numbers of eggs. At concentration of 0 – 4×10^3 spores, causing a strong decrease of female productivity from 102 eggs to 50 eggs respectively and the hatching rate of eggs reduced from 94.11% to 76.38% at the same concentrations. Also, the pre- oviposition period was affected, where it took 4 days with the control (untreated) but with the concentration of 4×10^3 spores it reached 7 day which considered as significant effect of the fungus. [18] illustrated that when *Ceratitis capitata* was treated with 1×10^2 - 1×10^6 of *Penicillium chrysogenum*, caused fertility decrease ranging from 8% to 66.1% relatively, and the female fecundity was concentration dependent, and the reduction of the egg hatching rate to reach 31.7%. Also [26] tested infection of *Ae. Aegypti* with *B. bassiana* observing the reduction 30% of mosquito- human contact by the fungal infection in the laboratory and the fecundity was reduced to 29.3% eggs for each female in its lifetime.

Table (4): The effect of *Beauveria bassiana* isolate on the *Culex* female's fertility, pre-oviposition period and percentage of egg hatching (which developed from treated eggs),

Concentration mg/ml	Pre- oviposition period(days)	No. eggs	% egg hatching
0	4	102	94.11
1	5.5	80	92.5
2	6**	65*	84.6*
3	6.75**	53**	79.24*
4	7***	50**	76.38**

V. REFERENCES

- 1-Yang, Y. C.; Lee, S. G.; Kim, H. K. and Lee, H. S. (2002). A piperidine amide extracted from *Piper longum* L. Fruit shows activity against *Aedes aegypti* mosquito larvae. *J. Agric. Food Chem.*, 50(13): 3765-3767.
- 2-Hemingway, J. and Ranson, H. (2000). Insecticide resistance in insect vectors of human disease. *Annu. Rev. Entomol.*, 45(1): 371-391.
- 3-Raymond, M.; Berticat, C.; Weill, M.; Pasteur, N. and Chevillon, C. (2001). Insecticide resistance in the mosquito *Culex pipiens*: what have we learned about adaptation?. *Genetica*, 113: 287-296.
- 4-Su, T. and Mulla, M. S., (1998). Ovicidal activity of neem products (Azadirachtin) against *Culex tarsalis* and *Culex quinquefasciatus* (Diptera: Culicidae). *J. Am. Mosq. Control Assoc.*, 14(2): 204-209.
- 5-Seccacini, E.; Lucia, A.; Harburguer, L.; Zerba, E.; Licastro, S. and Masuh, H. (2008). Effectiveness of pyriproxyfen and diflubenzuron formulations as larvicides against *Aedes aegypti*. *Journal of the American Mosquito Control Association*, 24(3):398-403.
- 6-Amiri, A.; Bandani, A.R. and Ravan, S. (2010). Effect of an anti-juvenile hormone agent (Precocene I) on *Sunn pest*, *Eurygaster integriceps* (Hemiptera: Scutelleridae) development and reproduction. *African Journal of Biotechnology*, 9 (36): 5859-5868.
- 7-Al-Chalabi, B. M.; Mehdi, M. T. and Al- Suhaily, I., A. (1984). The effect of Aflatoxin produced in stored products on the biology of *Callosobruchus maculatus* (Fab.). *J. Biol. Sci. Res.*, 15 (2): 13 – 28.
- 8-Al-Chalabi, B. M. and Mohsen, Z. H. (1989). The efficacy of different formulations and strains of Bacilli to *Culex quinquefasciatus* Say (Diptera: Culicidae) larvae at various exposure periods. *Proceedings of the fifth scientific Conference Scientific Research Council, Biological Sciences*, 7 – 11 Oct. 1989, Baghdad, Iraq. Vol. 5, part2: 125 –131.
- 9-Gillespie, A. T. and Claydon, N. (1989). The use of entomogenous fungi for pest control and the role of toxins in pathogenesis. *Pesticide Science*, 27(2):203- 215.
- 10-Boucias, D. R. and Pendland, J. C. (1998). Entomopathogenic fungi; Fungi Imperfecti. In: *Principles of Insect Pathology*. Edited by: Boucias D. R Pendland J.C., Kluwer Academic Publishers.
- 11-Scholte, E. J.; Knols, B. G. and Takken, W. (2006). Infection of the malaria mosquito *Anopheles gambiae* with the entomopathogenic fungus *Metarhizium anisopliae* reduces blood feeding and fecundity. *Journal of invertebrate pathology*, 91(1): 43-49.
- 12-Khna, S.; Guo, L.; Maimaiti, Y.; Mijit, M. and Qiu, O. (2012). Entomopathogenic fungi as microbial biocontrol agent. *Molecular Plant Breeding*, 3(1): 63-79.
- 13-Barbarin, A. M.; Jenkins, N. E.; Rajotte, E. G. and Thomas and Matthew, B. (2012). "A preliminary evaluation of the potential of *Beauveria bassiana* for bed control" (PDF). *Journal of Invertebrate Pathology* 111 (1): 82–85.
- 14-Mohsen, Z. M. and Mehdi, N. S. (1989). Effects of insect growth inhibitor alsystin on *Culex quinquefasciatus* Say (Diptera: Culicidae). *Anz. Schadlingskde. Pflanzenschutz, Umwelt schutz*, 61: 130-133.
- 15-Al-Chalabi, B. M. and Mones, A. H. (2006). The insecticidal affects of Ngos and Sumicidin against eggs and larvae of the mosquito *Culex pipiens* L. (Diptera: Culicidae). *Ibn AL-Haitham journal for pure and applied Sciences*, 19(4):34-40.
- 16-Abdulla I.T. (2002). Modulation of immune response in BALN/c mice against infection with hydatid disease by the polysaccharide extracted from *Pseudomonas aeruginosa*. M.Sc. Thesis College of Education. University of Mosul. Iraq.
- 17-Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18: 265-267.
- 18-Castillo, M. A.; Moya, P.; Hernández, E. and Primo-Yúfera, E. (2000). Susceptibility of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) entomopathogenic fungi and their extracts. *Biological control*, 19(3), 274-282.
- 19-Dinalva, M.; Antonio, M.; Ana, R. M. and Luciana, Y. (2009). Efficiency of entomopathogenic fungi in the control of eggs and larvae of the horn fly *Haematobia irritans* (Diptera: Muscidae). *Veterinary Parasitology* 167:62–66.
- 20-Bukhari, T., Willem, T., Constantianus, J. M., Koenraadt., (2011). Development of *Metarhizium anisopliae* and *Beauveria bassiana* formulations for control of malaria mosquito larvae. *Parasites & Vectors* 4:23.
- 21-Amóra, S. S. A., Bevilaqua, C. M. L., Feijó, F. M. C., Silva, M. A., Pereira, R. H. M. A., Silva, S. C., and Oliveira, D. M. (2009). Evaluation of the fungus *Beauveria bassiana* (Deuteromycotina: Hyphomycetes), a potential biological control agent of *Lutzomyia longipalpis* (Diptera, Psychodidae). *Biological Control*, 50(3), 329-335.
- 22-Clark, T. B., Kellen, W., Fukuda, T., and Lindgren, J.E., (1968). Field and laboratory studies on the pathogenicity of the fungus *Beauveria bassiana* to three genera of mosquitoes. *Journal of Invertebrate Pathology* 11: 1-7.

23-Bukhari, T., Middelma, A., Koenraadt, C. J. M., Takken, W., and Knols, B. G. J., (2010). Factors affecting fungus-induced larval mortality in *Anopheles gambiae* and *Anopheles stephensi*. *Malaria Journal*, 9:22.

24-Ekesi, S., Maniania, N. K., and Lux, S.A. (2002). Mortality in three African tephritid fruit fly puparia and caused by the entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana*. *Biocontrol Science and Technology* 12:7-17.

25-Angel-Sahagún, C. A., Lezama-Gutiérrez, R., Molina-Ochoa, J., Galindo-Velasco, E., López-Edwards, M., Rebolledo-Domínguez, and Foster, J. E. (2005). Susceptibility of biological stages of the horn fly, *Haematobia irritans*, to entomopathogenic fungi (Hyphomycetes). *Journal of Insect Science*, 5(1).5-50.

26-Darbro, J. M., Johnson, P. H., Thomas, M. B., Ritchie, S. A., Kay, B. H., and Ryan, P. A. (2012). Effects of *Beauveria bassiana* on survival, blood-feeding success, and fecundity of *Aedes aegypti* in laboratory and semi-field conditions. *The American journal of tropical medicine and hygiene*, 86(4), 656-664.

