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Efficacy of *Baeuvariabassiana* (Bals.) Vuil against *Chrysomyamegacephala* (Fabricius) Larvae (Diptera: Calliphoridae) via Contact and Ingestion

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Abstract: ***Pathogenicity of two isolates of Entomopathagenic fungus of Beauvariabassiana against Chrysomyamegacephala larvae were evaluated in the laboratory. This is the first experiment to evaluate the pathogenicity of a local isolate of this fungus against blow fly larvae. Two bioassay methods were conducted: spraying the larvae directly with fungalsporessuspension and mixing its food by three concentrations of spores suspensions (10^6, 10^7 and 10^8 spores.ml^{-1}).Data showed that the two isolates of B. bassiana 2 (Imported isolate) and B. bassiana 1 (Local isolate) were effective and caused mortality and malformation in the treated blow fly larvae. The results revealed that accumulative mortality percentage of the treated larvae by directly spraying by 10^8 conidia.ml^{-1} were 90 and 63.33 for B. bassiana 2 and 1 respectively.The results of mixing the larval food were 100% and 70% respectively, different morphological malformation were depicted such as shrinking and brownish the larval body. The high larval accumulative mortality percentages by the two treatment methods showed that this blow fly species was very sensitive against this fungus isolates, which show that these isolates are promising agents for controlling it.***

Keywords: *Chrysomyamegacephala*, *Beauvariabassiana*, Larvae.

1. Introduction

Chrysomyamegacaphala (Blow fly) is a species of Calliphoridae family that is associated with human health and pets because it is a vector of pathogens [1]. Because of its nature in living and breeding on animal carcasses, decomposing organic matter and animal and human excrements, they appear in large numbers around it, as well as can be seen around the exposed meat in the markets and slaughter houses [2, 3]. As a result, this species is a medically important carrier of pathogens (viruses, bacteria, pathogenic pathogens, and some nematodes) mechanically [4-6], as well as to the state of healing in wounds or in certain areas of the body in humans and animals [7-9]. The fly is also an economic pest that causes damage to fish that is dried under sunlight [10].

Microorganisms in the soil affect on insects naturally and may cause diseases that can lead to high mortality rates within insect community, these organisms often include bacteria and fungi, so these organisms have been used to eliminate many insect pests which has a specialized effect and its nature that is not effective in the environment and can be commercially produced as alternatives to harmful chemical pesticides environmentally [11].

Beauvariabassiana is Entomopathogenie fungi, this fungus represents the first species to be used as a bio pesticides, It has been known to be an effective control agent for many insect pests, causing white muscardine [11]. This type is characterized by its global spread as it is found naturally in the soil and on the bodies of insects cadaver and decomposed plants, due to its saprophytism nature as well as it can be active in nature for a long time [12, 13].

The infection of this insect occurs after contact and adhesion of spores with its body wall when the conditions are appropriate to growth and penetration of these spores, then fungus conidia grow and invade all organs, tissues and cavities of the insect body, after about 7-10 days, due to the fungus growth many enzymes and toxins produced such as Protease and Beauvercin followed by external growth of the fungus covering the entire insect body [14].*B. bassiana* has achieved excellent roles in controlling of many insect pests belonging to the Lepidoptera, Coleoptera and Diptera [15-17].

The aims of this research is to study the effect of two isolates of *B.assiana*, the first isolate was imported and the second isolate from the soil of Baghdad, the study was first conducted on fly larvae of the species *Ch. megacephala* because of its medical and economic importance.

2. Materials and methods

2.1 Collection and breeding of the insect

A collection of blow fly larvae (about 25 larvae) was collected from a piece of meat left in Baghdad area, then transported to insect Laboratory, Department of Biology at the College of Education for Pure Sciences Ibn al-Haitham, University of Baghdad, then placed in disposable plastic containers with 25 g of fat free minced beef, then it was put into a larger containers containing sterile a soft sawdust, the containers were placed in a metal box (30 x 30 x 30 cm) with a petri dish (9 cm diameter) contains sugar and dry milk (1 : 1) for feeding

insects, as well as there was another petri dish containing wet cotton, care and surveillance continued until the emergence of adults insects. The insect identification as *Ch. megacephala* was conducted by the Museum of Natural History and the Research Center in University of Baghdad, the insect was breed for three generations before doing the experiment.

2.2 Breeding of the two fungus colonies

Two isolates of the *B.assiana* fungus were used, one of which was imported by the Agricultural Research Center - Ministry of Science and Technology, marked with (*B. basiaina* 1) and the second was isolated from Baghdad area and marked with *B. Bassiana* 2.

Both isolates were placed on Potato Dextrose Agar medium (after adding chloramphenicol) in Petri dishes and incubated at a temperature of 27±2 ° C for 7-14 days.

2.3 Preparation of fungal suspension concentrations

Three suspension concentrations of both isolates (1 and 2) 10^6, 10^7 and 10^8 spore.ml^{-1} were prepared using sterile distilled water added to Tween-20 0.01% concentration to study the effect of these concentrations in the second instar larvae of blow fly type *Ch. megacephala*.

2.4 Biological experiments

The second instar larvae were treated with concentrations (10^6, 10^7, 10^8 spore.ml^{-1}) of both the 1 and 2 isolates by direct spraying. The method included transferring 10 larvae from the breeding colony to a disposable plastic container (its base diameter 4.5cm and its height 3.5 cm). The larvae were sprayed with about 2 ml of the isolates from a distance of 10-15 cm using a sterile hand spray, then the larvae were transferred to another container containing 10 g of fat free minced beef (which represents the special media for larvae feeding), the plastic container coveredby perforated covers.Each experiment includes three replicates of each concentration, while control treatment includes spraying the larvae with 0.01% concentration of Tween-20 with distilled water. The containers were left in an incubator at 27±2° C, humidity 80±5% and a 12-hour illumination period, the experiment was re-applied to the other concentrations.

The larvae were monitored daily to record the number of dead and deformed larvae, then the daily and cumulative mortality rates, survival rates, abnormalities, and adult emerge rates were calculated. The larvae and malformed insects were photographed by a dissecting microscope fitted with a camera [18].

The effect of the concentrations of *B. bassiana*isolates 1 and 2 was studied in the larvae *Ch. megacephala* as well as the method of treating larval food with the mentioned concentrations, the method was adding 10 gm of larval food (minced beef meat) with about 2 ml from the concentration 10^6 spore.ml^{-1} and mix well in a new plastic container, after that 10 larvae were transferred from the culture colony to the food, with a perforated cap, three replicates were applied to control treatment by adding 2 ml of 0.01% concentration of Tween-20 with distilled water, the experiment containers were placed in an incubator at 27 ±2 ° C, humidity 80±5% and 12-hour illumination, the containers were monitored daily to record the number of mortalities, deformities and adults emerged insects (18).

2.5. Statistical analysis

The percentages of mortality were corrected according to (Abbott's equation) [19], Subsequently, SAS [20] was used to data analysis which was the sensitivity of fungus concentrations in cumulative larval mortalities, deformity and adult emergency, the significant differences were compared between mean and least significant difference (LSD).

3. Results and Discussion

B. bassiana is characterized by its high ability to cause infection and kill its hosts of different species of insects, the results listed in table (1) show that the spraying of the second instar larvae of*Ch. megacephala* with different concentrations of spores of both isolates (imported and local) resulting an increase of cumulative mortalities percentages from 40.00-63.33 at 10^6 and 10^8 spore.ml^{-1} respectively with imported isolate 1 and 45.00-90.00 at the same levels respectively with local isolate 2, the results also refer to significant differences between the two isolates, the local isolate was superior compared with the imported one in their ability to infection in the blow fly larvae, which makes this isolate very good to be used as a bio control method.A high malformation percent was observed in the treated larvae (66.67% and 83.33%) at concentration 10^7 spore.ml^{-1} of the imported and local isolates respectively, these malformation were appeared as brownishing and darking the larval body and the fungal growth out of larvae, also when the non-dead larvae were followed until they were turned into adults. The insects appeared distorted, with narrow wings and the growth of the fungal spinning above them and die after only one day later. The results are in a good in agreement with those of Narladkar*et al.* [17], where they noted that the treatment of Culicoides' larvae by *C. bassiana* suspension resulted in their mortality, deformation and the emergence of conidia spinning on the resulting adults insects from treated larvae.

Table 1: Effect of concentrations of spores suspensions of *B. bassiana* 1 and *B. bassiana* 2 by direct spraying of *Ch. megacephala*larvae.

Isolate type	Concentration Spore.ml^{-1}	Cumulative mortality percent	Average of deformities percent	average of adults emergence percent
		Mean ± S.E.		
*B. bassiana*1	10^6	40.00±0.00^{AaC}	23.33±8.82^{Aac}	60.00±0.00^{AaC}
	10^7	63.33±6.67^{AaC}	66.67±8.82^{AaC}	36.67±6.67^{AaC}
	10^8	63.33±13.33^{AaC}	10.00±0.00^{Aac}	36.67±13.33^{AaC}
B. bassiana 2	10^6	45.00±2.89^{Aac}	53.33±8.82^{Aac}	55.00±2.89^{AaC}
	10^7	30.00±5.77^{Aac}	83.33±3.33^{AaC}	70.00±5.77^{AaC}
	10^8	90.00±5.77^{AaC}	60.00±11.55^{Aac}	10.00±5.77^{Aac}
control	0.00	6.67±3.33	0.00±0.00	93.33±3.33

a = there is significant differences at (P<0.05)compared to control treatment.
A = there is significant differences at (P<0.05) between types of isolates and concentration.
b = there is no significant differences at (P<0.05) compared to control treatment.
B = there is no significant differences at (P<0.05) between types of isolates and concentration.
C = there is significant differences at (P<0.05) with increase of concentration.
c = there is no significant differences at (P<0.05) with increase of concentration.

Other studies ,e.g. Mehinto*et al*. [15] tested seven isolates of *B. bassiana* and foud that they were it was effective in controlling the larvae of *Marucavitrata* and the mortality rate reached 67.5% at 10^8 spore.ml^{-1} of one of these isolates after 15 days of treatment, so that the results of this study, agree with the result mentioned [15].

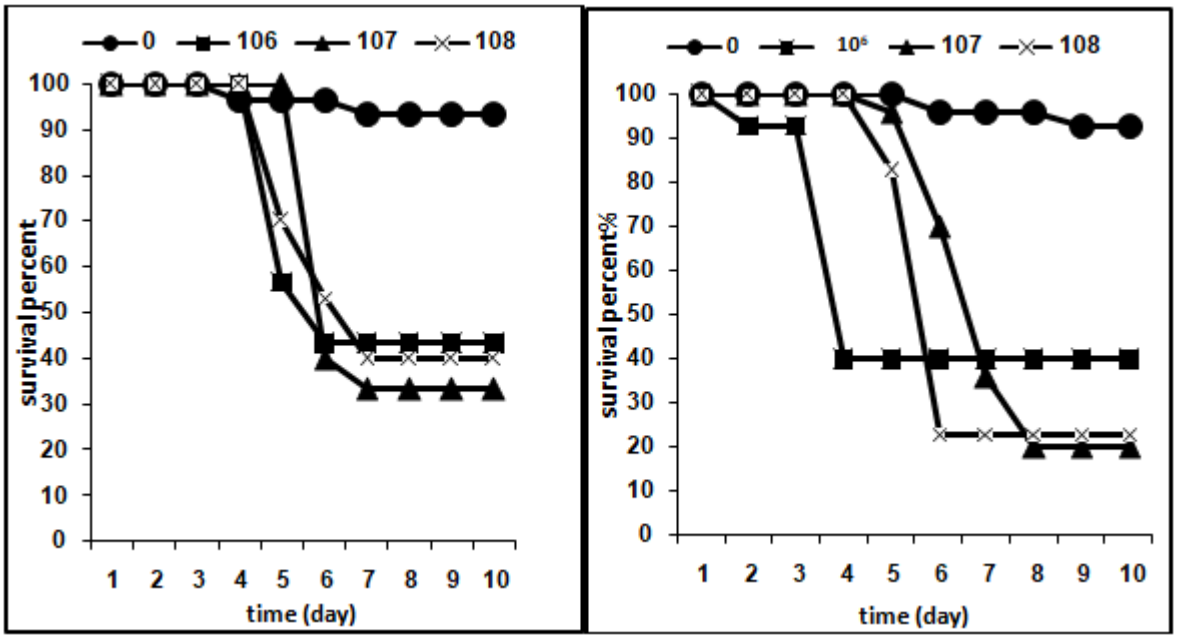
Fig (1) shows that the fungal infection reduced the survival rate of treated larvae by 40% after spraying with concentration 10^6 spore.ml^{-1} of the (1 and 2) isolates. This result is in good agreement with results of Mohammadbeigi and Port [21], where it was revealed that spraying the locusts *Uvarovistia zebra* at a concentration of 10^8 x 1.5 spore.ml^{-1} of the *B. bassiana* fungi suspension reduced its survival to less than 20% after 10 days of treatment.

Table (2) reflects that the treatment of blow fly larval food with *B. baciana* isolates (isolate 1 and isolate 2) resulted in high cumulative morality percent 100 and 70 at concentration 10^8 spore.ml^{-1} with local and imported isolates respectively, this resulted ina decrease of rate of adult emergency, as well as recorded high malformation and brownishing of dead larvae, and there were significant differences among the cumulative morality rates and isolates types of fungi and concentrations.

Fig (2) shows that the survival rates of the larvae that were feeding on the food treated by suspension of the two isolates decreased significantly by 10% at the concentration 10^7 spore.mL^{-1} of the local isolate suspension, while the rate was less than 40% of the imported isolate with the same concentration.

These results were in a good agreement with those of Salih*et al.* [22] which show the treatment of the early phase larvae of *Agrotisoipsillia* with a concentration of 10^7 spore.ml^{-1} of local isolation of *B. bassiana* resulted in a high mortality in laboratory conditions. In this regard, Sharififard*et al*. [23] showed that the treatment of domestic fly larvae (*Musca domestica*) with a concentration of 10^7spore.ml^{-1} of *B. bassiana* suspension resulted in a morality of 98.4%. The results we got are in agreement with those reported in literature [17], they indicated that the number of Culicoids changed only after seven days of treatment with fungi suspension of *B. bassiana*. Also this is in agreement with results of Gobarry*et al*. [24], who concluded that the treatment of second-instar larvae of *Agrotisipsilon* with a concentration 10^8 x 2 spore.ml^{-1} of *B. bassiana* suspension resulted in a morality after seven days and the emergence of fungus on bodies of dead larvae was after 13-10 days. The high mortality rates are attributed to the growth of fungi inside the digestive tract of the insect accompanied by the secretion of enzymes and toxins that lead to the destruction and decay of their bodies, the most important of these enzymes is the protease enzyme, which analyzes the complex protein molecules that are involved in building the tissues of the insect body, as well as the enzyme of Chitinase which analyzes the chitin material, which enters in formation of internal casing of the digestive system in insect body, and some toxins such as Beauvercin are followed by an external growth of conidia (the body of the dead insect) that carry many spores covering the body, which considered as a new source of infection [25].

The results of this study confirm the effectiveness of local and imported isolates in control of *Ch. megacephala*, which has a medical and economic importance, these isolates are important bio control agents. The dependence on local isolates is an effective factor in reducing the spread of insects and diseases vectors and reducing the use of insecticides which cause environmental pollution.



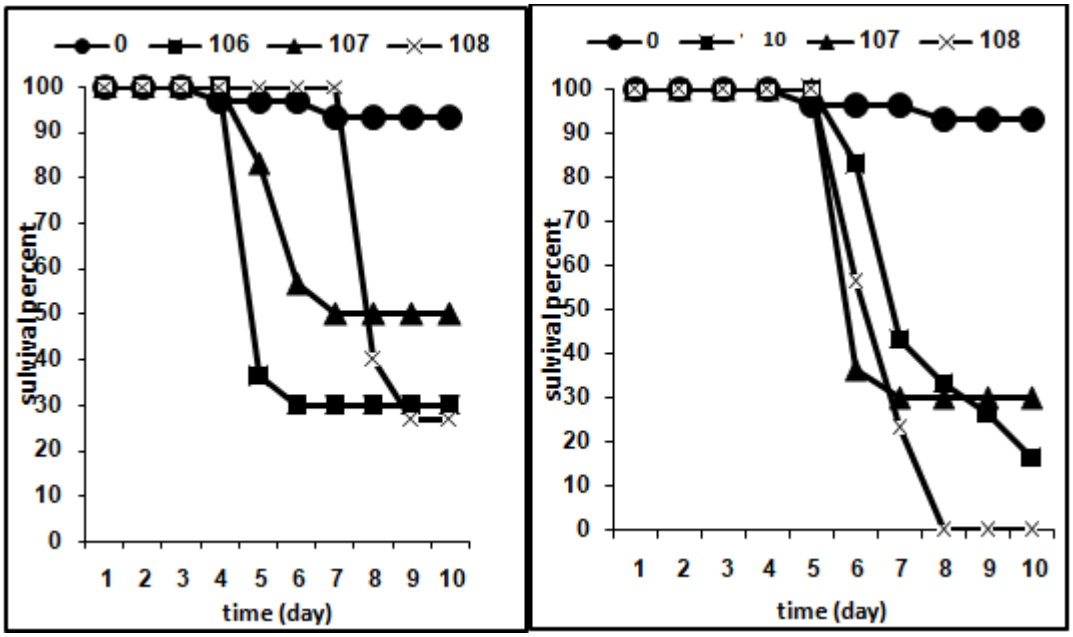
B. bassiana 1*B. bassiana* 2

Figure 1: Survival of blow fly larvae *Ch. megacephala*after spraying with different concentrations of fungi spores suspensions of*B. bassiana* (1&2).

Table 2: Effect of sequential concentrations of suspensions of *B. basiana* 1 and *B. bassiana* 2 by using feed treatment of the Second phase of larvae blow fly *Ch. megacephala*.

Isolate type	Concentration Spore.ml^{-1}	Cumulative mortality percent	Average of malformation percent	Average of adults emergence
		Mean ± S.E.		
*B. bassiana*1	10^6	40.00±0.00^{AaC}	23.33±8.82^{Aac}	60.00±0.00^{Aac}
	10^7	63.33±6.67^{AaC}	10±0.00^{Aac}	36.67±6.67^{Aac}
	10^8	70.00±0.00^{Aac}	13±3.33^{AaC}	30±0.00^{AaC}
*B. bassiana*2	10^6	65.17±2.89^{AaC}	41.83±9.22^{AaC}	34.83±2.89^{Aac}
	10^7	93.5±3.25^{Aac}	42.3±2.20^{AaC}	7.25±2.75^{Aac}
	10^8	100±0.00^{AaC}	40±11.55^{Aac}	*
control	0.00	6.67±3.33	0.00±0.00	93.33±3.33

a = there is significant differences at (P<0.05)compared to control treatment.
A = there is significant differences at (P<0.05) between types of isolates and concentration.
b = there is no significant differences at (P<0.05) compared to control treatment.
B = there is no significant differences at (P<0.05) between types of isolates and concentration.
C = there is significant differences at (P<0.05) with increase of concentration.
c = there is no significant differences at (P<0.05) with increase of concentration.
*= mortality of all treated larvae.



***B. bassiana* 1** ***B. bassiana* 2**

Figure 2: Survival of blow fly larvae *Ch. megacephala* after treatment of feed with *B. bisiana* (1 and 2) in different concentrations

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