

ROLE OF CERTAIN *BEAUVERIA BASSIANA* (BALS.) ISOLATE AS BIOLOGICAL CONTROL AGENT AGAINST COWPEA APHID (*Aphis craccivora* koch) AND ITS EFFECT ON THE PREDATOR *Chrysopela carnea* (STEPHENS).

Imam, M. I¹. and Iman I. Imam²

1-Entomology Department, Faculty of Science, Ain Shams University.

2-Economic Entomology Unit, Plant Protection Department, Desert Research Center.,

ABSTRACT

The lethal effect of *Beauveria bassiana* isolate on *Aphis craccivora* revealed that the least percentage of adult mortality was 11%, which was recorded with the lowest tested concentration (1×10^3 spores/ml), while The highest percentage of adult mortality was (54%) was achieved at (1×10^7 spores/ml). compared with 4% in natural mortality. In a similar trial to evaluate the effect of *B. bassiana* against the aphid lion *Chrysopela carnea* (Stephens), exposure of the 2nd larval instar of aphid lion to *B. bassiana* isolate either through direct or indirect exposure resulted in adverse effects on the total percent of larval mortality, which was concentration dependent. Whereas the total larval death recorded 3% in control trials, data in case of direct exposure revealed 9 to 41% of larval death at 1×10^3 and 1×10^7 spores/ ml, respectively, Comparable trend was recorded with larvae indirectly exposed. A slight increase in larval mortality was recorded by 5 to 15 %, respectively. In case of direct exposure technique the hatchability percentage decreased by increasing fungal concentration. The fluctuation in the indirect exposure technique did not statistically differ from that recorded in case of control trials.

INTRODUCTION

Natural enemies play a very important part in controlling pest populations. Green lacewing *Chrysoperla carnea* (Stephens), known as an insect in the Chrysopidae family. The adults feed on nectar, pollen and aphid honeydew and are not predatory, but the larvae are efficient biocontrolling predators against many key pests including aphids, whiteflies, young larvae and eggs of Lepidoptera, mealybugs, spider mites and soft bodied arthropods (El- Arnaouty & Ferran 1993 and Hassan 2003). It can be mass reared in the laboratory and released against pests in field and green houses (Mirnoayedi, 2001).

Aphis craccivora cowpea aphid (Order / Homoptera: Family Aphididae) or plant lice, is one of the hundreds of different species of aphids. They are small soft-bodied insects some of which attack only one host plant while others attack numerous hosts.

Aphids feed by sucking up plant juices and at the same time, inject saliva into the host. Light infestations are usually not harmful to plants, but higher aphid infestations may result in leaf curl, wilting, stunting of shoot growth, and delay in production of flowers and fruit, as well as a general decline in plant vigor. Some aphids are also important vectors of plant diseases, transmitting pathogens in the feeding process.

Fungal microbial control agent offer a method of insect pest control that can be integrated with other biocontrol agents, *Beauveria bassiana* (Bais.) was ubiquitous as an entomopathogen of a wide range of insect's from most insect orders (Goettel and Jaronski, 1997). However, the success of fungal entomopathogens as biological control agents depends not only on high efficacy against insect pests, but also on low virulence against non target insects. Therefore the current investigation aims to study the role of certain *B. bassiana* isolate a biological control agent against *A. craccivora* and its effect on the chrysopid predator *C. carnea*.

MATERIALS AND METHODS

1- Origin and maintenance of insect culture:

A. craccivora kock (Homoptera: Aphididae) feed on bean plants (*Vicia faba*) by sucking plant juices. Numbers of pots were planted with seeds of bean. When the plant grew above the soil, artificial infestation was achieved by transferring heavily infested leaves to the new plants. Aphids were transferred weekly from old to young plants. The colony was maintained under laboratory conditions.

Rearing was carried out in a box, two sides of which were made up of wire mesh, the upper part was made up of glass, and the rest was wood.

The predator, *C. carnea* was initially collected from the cotton field and reared on Angoumois grain moth, *Sitotroga cerealella* (Olivier) at the same mentioned laboratory conditions. The adults of *C. carnea* were sexed and 10 pairs of adults were placed in plastic boxes (22x13x10 cm) covered with black muslin for deposited eggs and changed daily. Drops of Semi artificial diet solution consists of 2g yeast extract, 1g fructose and 1cm distilled water were provided on tape stacked on the muslin cover. The deposited eggs were collected daily and kept in glass jars until hatching. The hatched larvae were reared on *S. cerealella* eggs. (Hassan and Ezz 2009).

The original colony of the predator and its prey were supplied from the Plant Protection Research Institute, Agriculture Research Centre. Mass rearing was carried out in the laboratory of the Economic Entomology Unit, Plant Protection Department, Desert Research Center.

2-Fungus culture:

The fungal isolate *B. bassiana* was isolate from soil samples by Abd El Nasser A. M. Kobisi, Economic Entomology Unit, Plant Protection Department, Desert Research Centre. By using the soil plate method (Warcup, 1950). Both light and electron microscopy were used for examination and identification of the fungal isolate according to (Raper and Fennel, 1965) and (Samson *et al.*, 1995). The *B. bassiana* isolate was cultured on liquid medium after purification by sub- culting on potato dextrose agar (PDA) medium. One disc contain spores was cut from edge of actively growing culture and inoculated under aseptic condition in each sterilized media (adjusted at pH 6.5)

of Potato dextrose broth (PDB 50 ml) medium in Erlenmeyer flask (250 ml capacity). The fungal isolate was transferred to an incubator maintaining $28 \pm 2^\circ\text{C}$. After 14 days of incubation period the mycelial mat of isolate was harvested, washed with distilled water for several time, the extract by refluxing in boiled methanol for 2 hours and then filtered off. The mycelial residue was reextracted again for three times. The combined filtrates were concentrated under reduced pressure at temperature not exceeding 35°C . The obtained residue was kept in refrigerator for investigation against the target insect. The filter of isolate was extracted by *n*-butanol. This step was repeated until complete extraction. The butanolic extract was filtered on anhydrous of sodium sulphate. Fungal suspension concentrations were adjusted by estimation on a haemocytometer (hirschmann $0.1 \times 0.0025 \text{ mm}^2$).

3-Toxicity Test:

A-Method of application on Aphid:

To determinate the effect of the entomopathogenic fungus on *A. craccivora*, assay was carried out on adult (2-3 days old). Three concentrations (1×10^3 , 1×10^5 and 1×10^7 spores / ml) of fungal suspension were used. Treatments and control represented with 3 replicates and each replicate consisted of ten adults of *A. craccivora*. Each replicate was sprayed with 1ml of fungal suspension in small plastic cages then transferred to 9 cm Petri dish. Control was treated with water. Daily mortality rates were noted and dead adults were monitored for mycosis symptoms. Data were analyzed for determination of the lethal concentration (LC_{50}).

B-Method of application on *C. carnea* larvae:

B.1- Direct effect:

The residual film technique was used. 3 ml of the desired concentration were evenly spread on a Petri dish surface (9 cm in diameter). The solvent allowed being evaporated leaving a film of several concentration of fungal isolate (1×10^3 , 1×10^5 and 1×10^7 spores / ml) the 2nd larval instar of aphid lion were exposed to the thin film for 24 hour and offer them the appropriate amount of Aphid as food. The concentration was replicated 6 times; 5 petri dishes for each containing three larvae. The control specimens were treated with water.

B.2- Indirect effect:

The second larval instar of aphid lion, were fed on larvae of Aphid previously sprayed with the previous concentrations of fungal isolate, then immediately placed with 2nd instar larvae of aphid lion. The control specimens were treated with water. Daily inspections were conducted.

C- Statistical Analysis and Assessment of Results:

Data obtained in different tests were subjected to statistical analysis to evaluate the relative efficiency of the isolate. Mortalities were corrected for the natural mortality according to (Abbot's formula, 1925).

The corrected percent = (Observed %-Control %) x 100/ (100-Control %)

Concentration / mortality regression lines were drawn on probit logarithmic graph according to the method developed by (Finney, 1971).

The LC_{50} and LC_{90} values were calculated according to probane

program.

percent of hatchability of the resulted females were calculated according to (Zidan *et al.*, 1998) as follows:

$$\% \text{ Hatchability} = [\text{No. of hatched eggs} / \text{No. of deposited eggs}] \times 100$$

RESULTS AND DISCUSSION

1- Toxic effect of *B. bassiana* isolate:

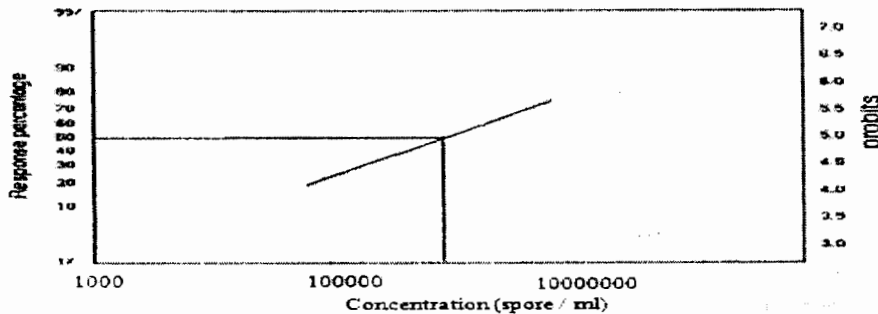
A-Effect on *A. craccivora*:

The lethal effect of *B. bassiana* isolate on *A. craccivora* adults was recorded. As shown in Table (1), the least percentage of adult mortality was 11%, which was recorded with the lowest tested concentration (1×10^3 spores/ml), while The highest percentage of adult mortality was (54%) was achieved at (1×10^7 spores/ml), compared with 4% in natural mortality.

Table (1): Toxic effect of *B. bassiana* isolate on 2-3 days old adult of *Aphis craccivora*.

Cocn. spores/ ml	Mortality % after 3days	
	Obs.	Corr.
0	4	0
1×10^3	11	7.29
1×10^5	26	22.91
1×10^7	54	52.08

The effect of *B. bassiana* isolate on *A. craccivora* adults could be detected on the basis of the calculated LC_{50} and LC_{90} values, which recorded 73868×10^6 and 83384×10^{10} spores/ml, respectively (Fig 1).



Fig(1): Concentration / mortality regression lines for *Aphis craccivora* adults treated with *B. bassiana* isolate

According to the recorded data all applied concentrations *B. bassiana* isolate reduced the population of *A. craccivora* adults. (Griffin, 1994) stated that the following fact; fungi secret wide array of compound that are biologically active against other organisms. (Goettel and Jaronski, 1997) decided *B. bassiana* was ubiquitous as an entomopathogen of a wide range of insects from most insect orders. It is recorded and used in suppressing population of several economically important insects including aphids, whiteflies, mealybugs, lepidopteran eggs and mites

including aphids, whiteflies, mealybugs, lepidopteran eggs and mites (Pena *et al.*, 1996; Vandenberg *et al.*, 1998; and Ezz, 2004). These record death percentages may be attributed to paralysis (mouthpart or midgut) and /or cytotoxin effect (Maketon *et al.*, 2008).

B- Effect on *C. carnea* larvae:

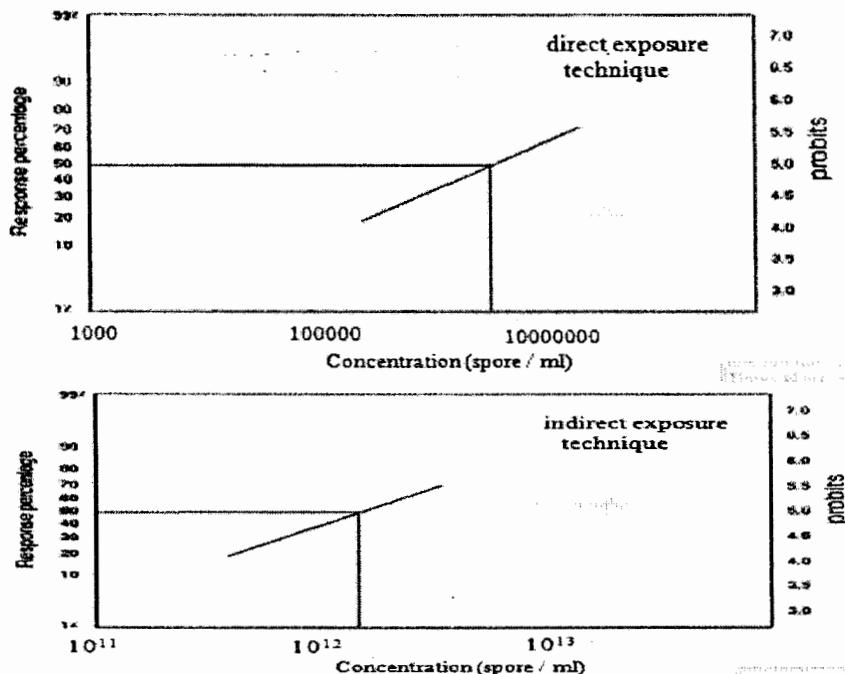
Exposure of the 2nd larval instar of aphid lion to *B. bassiana* isolate either through direct or indirect exposure procedure revealed adverse effects on the total percent of larval mortality, which was concentration dependent. Whereas the total larval death recorded 3% in control trials, data in case of direct exposure revealed 9 to 41% of larval death at 1×10^3 and 1×10^7 spores/ ml, respectively, Comparable trend was recorded with larvae indirectly exposed. A slight increase in larval mortality was recorded by 5 to 15 %, respectively (Table 2), Such mentioned data show that, although the tested *B. bassiana* isolate had the same mode of action in both exposure techniques the drastic larval death in the direct exposure method may be attributed to that the concentration of *B. bassiana* isolate, exceeded that in case of indirect method.

The direct effect of 2nd larval instar of aphid lion to *B. bassiana* isolate, could be detected on the basis of the LC₅₀ and LC₉₀ values which recorded 96279×10^7 and 6935×10^{11} spores/ ml, respectively, while the calculated LC₅₀ and LC₉₀ values of indirectly exposure technique recorded 10916×10^{12} and 92341×10^{18} spores/ ml, respectively, (Fig 2). The result revealed that indirect exposure technique was more effective than direct exposure technique. The data indicated that direct exposure technique was more effective than indirect exposure technique.

A novel bioassay has been developing that allows direct exposing *C. carnea* 2nd larval instar to the tested entomopathogenic fungus *B. bassiana* under laboratory condition. LC₅₀ of *B. bassiana* treated isolate is > the highest concentration tested. That is in agreement to demonstration of (Thungrabeab and Tongma, 2007) who assumed that *B. bassiana* was to be non pathogenic to *Chrysopela externa* (Hagen) with highest fungal concentration. In disagreement with that, (Sewify and Arnaouty, 1998) found harmful effects of two isolates of *Verticillium lecanii* on *Ch. carnea* larvae with significant difference between pathogenicity of the isolates. Nevertheless; different genera, species or strains of entomopathogenic fungi had different pathogenicity and virulence (Goettel, 1995 and Thungrabeab & Tongma, 2007).

Table (2): Toxic effect of *B. bassiana* isolate on 2nd instar larvae of *C. carnea*

Cocn. spores/ ml	Mortality % after 3days			
	Direct effect		Indirect effect	
	Obs.	Corr.	Obs.	Corr.
0	3	0	3	0
1×10^3	9	6.187	5	2.06
1×10^5	19	16.49	9	6.18
1×10^7	41	39.17	15	12.37



Fig(2): Concentration / mortality regression lines for aphid lion larvae treated with *B. bassiana* isolate

Chrysopela carnea egg hatchability was affected with treatment of to *B. bassiana* isolate (Table 3), (Fig. 3). In case of direct exposure technique the hatchability percentage decreased by increasing fungal concentration from about 70.86 to 36.14% at 1×10^3 to 1×10^5 spores/ ml, respectively, compared with about 91.67% in control females. The fluctuation in the indirect exposure technique did not statistically differ from that recorded in case of control trials.

Table (3): Hatchability percent of *C. carnea* eggs treated by *B. bassiana* isolate

Conc. spores/ ml	Egg number		Hatchability %	
	direct effect	indirect effect	direct effect	indirect effect
0	288		91.67	
1×10^3	278	280	70.86	90.27
1×10^5	226	278	61.06	91.23
1×10^7	166	275	36.14	90.01

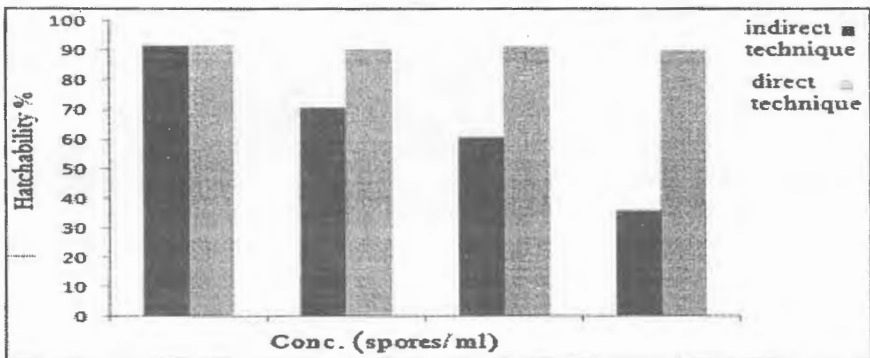


Fig. (3): Percentage of egg hatchability of *Ch. carnea* eggs treated by *B. bassiana* isolate

Direct effect caused decreasing in hatchability of Aphid lion eggs with increasing fungal concentration, discrepantly, Pessoa *et al.*, (2005) found innocuous effect of *B. bassiana* on *C. externa* eggs viability.

Sanehdeep *et al.*, (2011) tested the virulence of *B. bassiana* was against second, third and 4th instar larvae of *Spodoptera litura* (Fab.) using and noticed that, The eggs descended from treated larvae showed significant decrease in hatchability. Treatment of spiralling whitefly by entomopathogenic fungi, *B. bassiana* caused minimum egg hatchability as compared to control Boopathi Thangavel *et al.*,(2013)

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دور العازلات الفطرية (*Beauveria bassiana* (BAIS.) كمستحضر مكافحة بيولوجية على من القول وكذلك اثره على المفترس أسد المن *Chrysopela carnea*

محمد إبراهيم إمام^١ و إيمان إبراهيم إمام^٢

١- كلية العلوم - قسم علم الحشرات - جامعة عين شمس

٢- مركز بحوث الصحراء - قسم وقاية النباتات - حشرات إقتصادية

أوضحت النتائج المتحصل عليها من التجريب المعملی ان تأثير عازلات الفطر *Beauveria bassiana* على الحشرات البالغة من المن عند تركيز (1×10^3 spores/ml) حقق نسبة موت لا تتعدى ١١% ، على الجانب الآخر ارتفعت هذه النسبة من موت الحشرات الكاملة الى ٥٤% عند استخدام تركيز أعلى (1×10^7 spores/ml) ، في حين كانت نسبة الموت في المقارنة ٤% ، أما في حالة تجريب العازلات على يرقات العمر الثاني من المفترس اسد المن معمليا لتقييم اثره فكانت النتائج متباينة وفقا للتركيز المستخدم سواء بالتعرض المباشر او غير المباشر ، بلغت نسبة الموت في يرقات العمر الثاني لأسد المن في حالة التعرض الغير مباشر للعازلات الفطرية *B. bassiana* (التغذية على من معامل بالفطر) الى ٣% ، أما في حالة تعرض يرقات العمر الثاني لأسد المن مباشر للعازلات الفطرية تراوحت نسبة الموت لليرقات من ٩- ٤١% وفقا للتركيز المستخدم ، مما يستلزم الحرص في استخدام هذه العازلات الفطرية *B. bassiana* في حال استخدام مفترس اسد المن في برامج مكافحة المتكاملة وتجنب استخدامها في حالات اطلاق يرقات اسد المن .