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THE TOXIC EFFECT OF *BEAUVERIA BASSIANA* FUNGUS AND *NERIUM OLEANDER* PLANT EXTRACT AGAINST COWPEA APHID, *APHIS CRACCIVORA* KOCH (HOMOPTERA: APHIDIDAE)

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ABSTRACT: *The Cowpea aphid, Aphis craccivora Kock, is a common pest of several important plants. The toxic effects of different concentrations of the fungus Beauveria bassiana spores, and different concentrations of the leaf extract of Nerium oleander against Aphis craccivora Kock adults was calculated. The obtained results showed that the highest mortality percentages of aphid adult stages was 60 % recorded at the treatment of B. bassiana (1x10^{7}spores/ml) , while it was 91 % for the treatment of Nerium oleander leaf extract (2000 ppm). The lowest reduction percentages of aphids were recorded at the treatments of B. bassiana at the rate of (1x10^3 spores/ml) and the treatment of Nerium oleander leaf extract at the rate of (100 ppm) resulting only 13%.*

Key words: *Cowpea aphid, Aphis craccivora, Beauveria bassiana, Nerium oleander*

INTRODUCTION

Aphis craccivora Kock Cowpea aphid (Order: Homoptera: Family Aphididae) or plant lice, is one of the hundreds of different species of aphids. The aphids are insects of small soft-bodied, had a great wide of plant hosts, some attack only one host plant species while others attack several 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 heavy 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 aphid species 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* was ubiquitous as an entomopathogenic against a wide range of insects (Goettel and Jaronski, 1997). However, the success of entomopathogenic fungi as biological control agents depends not only on high efficacy against insect pests, but also on low virulence against non target insects.

Nerium oleander , commonly known as oleander, is an evergreen shrub or small tree in the dogbane family Apocynaceae, potentially toxic in all its parts. Plants of the previous Family are known to contain compounds producing major effects on insect behavior and physiology and insecticidal and repellent activity in *Nerium oleander* (El-Lakwah *et al.,* 1996). Oleander plant contains a toxin called Cardenolide Glycosides (Suganya *et al.,* 2012). This toxin is mostly found in the sap of the plant which is characterized by its clear to slightly milky colored and sticky. When ingested in certain quantities, this toxin can cause harm and possibly death.

The present work aims to conduct some toxicological studies on Cowpea aphid *, Aphis craccivora* using *Beauveria bassiana* spores and *Nerium oleander* leaf extracts.

MATERIALS AND METHODS

1- Tested insect:

The original colony of *Aphis craccivora* Kock (Homoptera: Aphididae) was supplied from the Aphid Research Department, Plant Protection

Research Institute, Agriculture Research Centre. Mass rearing of aphid insect was carried out in the laboratories of the Economic Entomology Unit, Plant Protection Department, Desert Research Centre.

The insects feed on bean plants (*Vicia faba*) by sucking plant juices. Seeds of beans were planted in rearing pots. When the plants grew above the soil (7 days), 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 25 ± 2 Cº and 70 % relative humidity RH.

Rearing pots were located in a wooden box with four sides of wire net and the upper side was made up of glass,

2- Fungus culture:

Beauveria bassiana fungus was isolated from soil samples by Abd El Nasser A. M. Kobisi at the laboratory of Economic Entomology Unit, Plant Protection Department, Desert Research Centre) using the soil plate method (Warcup, 1950). Stereoscopic microscope was used to examine and identify the isolated fungi according to (Raper and Fennel, 1965) and (Samson *et al*., 1995). The isolated *B. bassiana* 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 ±2˚C. After 14 days of incubation period the mycelia mat of isolate fungus was harvested, washed with distilled water for several times, and extract by refluxing in boiled methanol for 2 hours and then filtered off. The residual mycelia were re-extracted 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 (Hirscmann 0.1 x 0.0025 mm2).

3- Plant extract preparation:

The fully matured leaves of Oleander , *Nerium oleander* were collected from the farm of Desert research center. The leaves were dried in the shade for a period of 10 days. These leaves were blended into fine powder using electric blender. Ten grams of leaf powder was mixed with 100 ml of Acetone as solvent. The initial weight of the beaker is noted and the extraction was run in Soxhlet apparatus continuously for 2 hours and the final extract was collected in a beaker. The solvent is allowed to evaporate in hot air oven at 121˚C till complete evaporation was achieved. The final weight (initial weight gives the weight of leave extract) was stored until using. Two mg of extract was weighted and diluted in 20 ml of distilled water and use as standard, and was stored in dark bottle until using (Suganya *et al.,* 2012).

4- Assay method:

To determine the effect of the *Beauveria bassiana* isolated on the adults of *A. craccivora* (2-3 days old), three concentrations ($1x10^3$, $1x10^5$ and $1 x 10^7$ spores / ml) of fungal suspension were used. Three replicates (10 adults of *A. craccivora*) of each treatment were sprayed with one ml of fungal suspension in small plastic cages then transferred to Petri dishes (9 cm). Control treatment was sprayed with water only. Daily mortality rates were recorded and dead adults were monitored by mycosis symptoms. Data were analyzed for determination of the lethal concentration (LC_{50}).

As the previous method, the toxic effect of Oleander extract on *A. craccivora* adults was determined. Four concentrations (100, 500, 100 0 and 2000 ppm) of Oleander extract were used.

5- Statistical analysis and assessment of results:

- The obtained results in different treatments were subjected to statistical analysis to evaluate the relative efficiency of the isolates. Mortalities were corrected for the natural mortality according to (Abbott's formula, 1925):

Corrected mortality%= (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.

RESULTS AND DISCUSSION

1- Toxic effect of *B. bassiana* fungus on Aphid:

The lethal effect of *B. bassiana* isolated on *Aphis craccivora*, adults was recorded. As shown in Table (1), the least percentage of adult mortality (13%) was recorded with the lowest tested concentration ($1x10^3$spores/ml), while The highest percentage of adult mortality (60%) was achieved at ($1x10^7$spores/ml), in comparison with 6% in the treatment of control as natural mortality.

The toxic effect of *B. bassiana* fungus on *A. craccivora* adults could be detected on the basis of the calculated LC_{50} and LC_{90} values, which recorded 83699 x 10^6 and 89672 x 10^9 spores/ml, respectively (Fig 1).

According to the recorded data all applied concentrations of *B. bassiana* fungus reduced the population of *A. craccivora* adults at different degrees. (Griffin, 1994) stated that the toxic effect of entomopathogenic fungi due to that fungi secret wide array of compounds which are biologically active against other organisms. (Goettel and Jaronski, 1997) reported that *Beauveria bassiana* (Balsamo) was ubiquitous as an entomopathogenic against a wide range of insects. *Beauveria bassiana* was used in suppressing population of several economically important insects including aphids, whiteflies, mealybugs, lepidopteron eggs store insects and mites (Naqvi and Parveen, 1991; El-Lakwah ,1996 ; Pena *et al*., 1996 ; Vandenberg *et al*., 1998; and Ezz, 2004). Maketon *et al*.,2008 reported that the death of aphids treated with *Beauveria bassiana* fungus may be attributed to paralysis (mouthpart or midgut) and /or cytotoxin effect.

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

Ave no of spores/ ml	Mortality % after 3days	
	Observed	Corrected
control	6	0
$1x10^3$	13	7.45
$1x10^5$	29	24.47
$1x10^7$	60	57.45

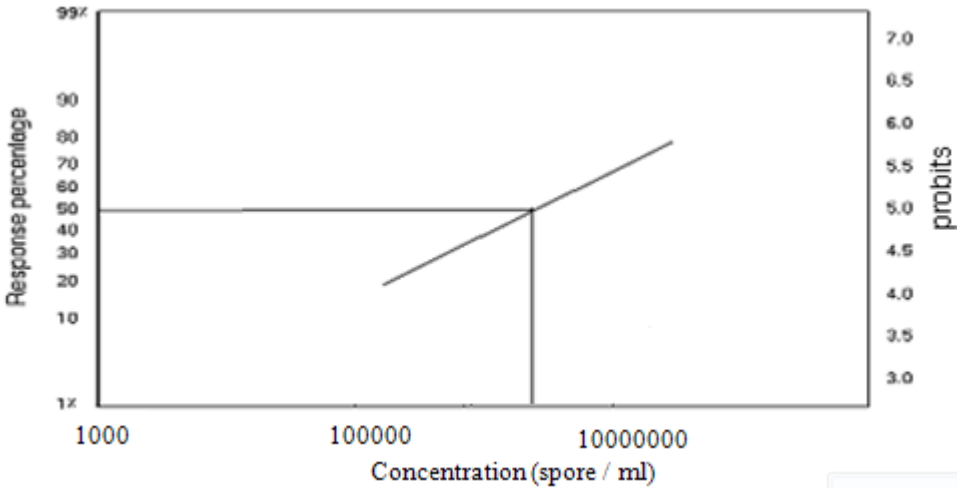

Fig (1): Concentration / mortality regression lines for *Aphis craccivora* adults treated with *B. bassiana* fungus

2- Toxic effect of Oleander leaves extract on aphid:

The lethal effect of *Nerium oleander* leaf extract on *A. craccivora* adults at different concentrations was evaluated. As shown in Table (2), the least reduction percentage of adult aphids was recorded at the lowest tested concentration (100 ppm) giving only 13%, while the highest reduction percentages of adult aphid stages was achieved at 2000 ppm recording 91% in comparison with only 10% as natural mortality at control treatment.

The effect of Oleander leaves extract on *A. craccivora* adults could be detected on the basis of the calculated LC_{50} and LC_{90} values, which recorded 710.02 and 2377.01 ppm (Fig 2).

Twenty four hours after treatment, it had been noted that the whole fungi with the aforementioned concentrations of Oleander extract (100, 500, 1000 and 2000 ppm) were died, therefore, from this phenomenon , the *N. oleander* leaf extract and *B. bassiana* isolate fungi must be used separately against insect pests. The ethanolic and acetone extracts of *Nerium indicum* and *Thuja orientelis* have been studied against III instar larvae of *A. stephensi* and *C. quinquefasciatus* (Sharma *et al*., 2005); the aqueous, lyophilized boiled water and ethanolic bark extracts of *N. indicum* was tested for the toxic effect against *Lymnaea acuminata* (Singh and Singh 1998); the toxic effects of crude extract was tested against *Tribolium castaneum* (Naqvi and Parveen 1991).

These results are in harmony with those of Hadizadeh *et al.,* 2009 who found high effective inhibition of *Nerium Oleander* leaves water extract against entomopathogenic fungi (*Fusarium oxysporim , F. solani, Rhizoctonia solani* and *Alternaria alternate*). Similar results are also recorded by (Phalisteen *et al*., 2008 and Osman *et al.,* 2007) who tested the effectiveness of water extract of *Nerium Oleander* leaves against *Posta placenta* and *Trametes versicolor.*

3- Toxic effect of extract of oleander leaves on fungal isolate:

Laboratory study was carried to examine the effect of water extract of *Nerium oleander* leaves against fungi isolate. Four concentrations of plant extract (100, 500, 1000, 2000 ppm) were used to determine their inhibitory effect on efficient of fungi on *A. craccivora*. Results showed complete inhibition activity on lethal effect of fungal isolate on the pest, where all tested concentrations of plant extract prevent the germination of fungus spores .

Table (2): Toxic effect of different concentration of Oleander leaves extract on 2-3 days old adult of *Aphis craccivora*.

Concentration (ppm)	Mortality % after 3days	
	Observed	Corrected
control	10	0
100	13	3.3
500	39	32.2
1000	65	61.1
2000	91	90

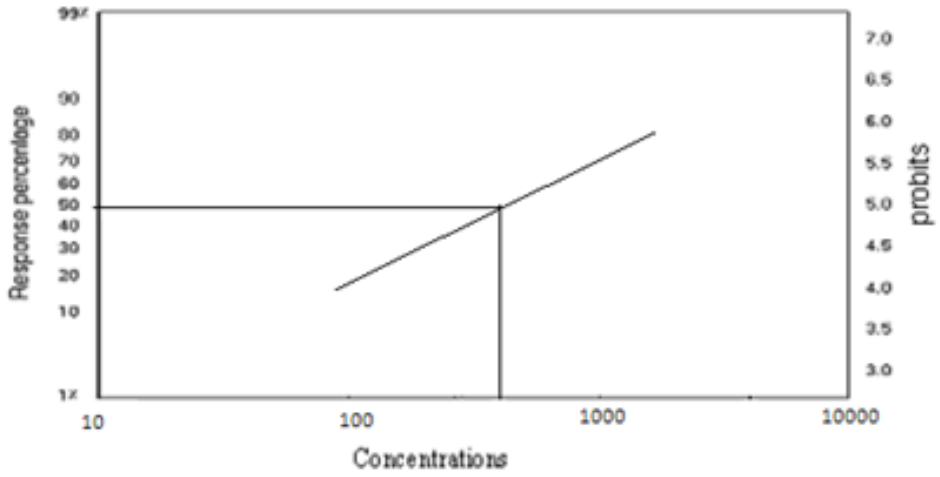

Fig. (2): Concentration/mortality regression lines for *Aphis craccivora*. adults treated with extract of Oleander leaves.

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التاثير المميت لفطر بوفيريا باسيانا ونبات الدفلة على حشرة مّن الفول

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الملخص العربي

تعتبر حشرة المّن من الآفات الخطيرة التي تسبب أضرار جسيمة للمحاصيل الزراعيه الاقتصادية. وفي هذه الدراسه تم إختبار التأثير المميت لعدة تركيزات من كلا من الفطر من نوع بيوفيريا باسيانا *Beauveria bassiana* والمستخلص المائي لأوراق الدفلة الطازجة علي الطور البالغ لحشرة مّن الفول ، وقد أسفرت النتائج أن أعلي نسبه مئوية لموت الحشرات الكامله بلغت ٦٠٪ عند تركيز (١x ١٠ ^{٧} جرثومة / ملل) بالنسبه للفطر ، ٩١٪ عند تركيز ٢٠٠٠ جزء فى المليون لمستخلص أوراق الدفلة بينما كانت النسبة المئوية للموت فقط ١٣٪ عند معاملة الحشرات بجراثيم الفطر بتركيز (١x ١٠ ^{٣} جرثومة / ملل) ومستخلص أوراق الدفلة عند تركيز ١٠٠ جزء فى المليون.

كما تم تحديد التركيز المميت لنصف المجموع (LC_{50}) هو ٨٣٦٩٩ x ١٠ ^{٦} جرثومة / ملل في حالة المعاملة بالفطر وقدر .710.02 ppm أما في حالة المعاملة بالمستخلص النباتي فقد قدر ال LC_{50} ب ٧١٠.٢ جزء فى المليون.

وقد وجد أن المستخلص المائي لأوراق نبات الدفلة له فعالية تثبيطية عالية علي التأثير السمي للفطر علي الحشرة فى جميع تركيزاته (من ١٠٠ الى ٢٠٠٠ جزء فى المليون) لذا يجب مراعاة عدم خلط واستخدام الفطر والمستخلص النباتي في برنامج مكافحة واحد.