Influence of Entomopathogenic Fungus *Beauveria bassiana* on the Mortality, Reproduction and Enzyme Activity of the Aphid Adults *Aphis craccivora* (Koch).

Wafaa L. Abdou, Elham A. Abdel-Hakim and Hala, M. Metwally

*Pests & Plant Protection Department, National Research Centre, Dokki-Cairo, Egypt*

**Received:** 18 July 2017 / **Accepted:** 28 August 2017 / **Publication date:** 10 Sept. 2017

**ABSTRACT**

Entomopathogenic fungi are recognized biological control agents of insects. The aim of the present study is to determine the effect of *Beauveria bassiana*, an entomopathogenic fungus against aphid *Aphis craccivora* (Koch), when treated in directly (plant treatment) or directly spray (aphids). Bioassay studies and enzymatic activity were carried out at three different concentrations of *B. bassiana*. We observed the positive effect of *B. bassiana* on mortality, reproduction (Neonate) and the enzymes (Phenoloxidase, peroxidase, Acetylcholine esterase and α-Amylase) activity in *A. craccivora*. After four days of direct treatments, all fungal concentrations caused significant mortality ranged between 26.67 and 80% in (*Aphis craccivora*). While the results of indirect treatments show an increase in mortality percentage from day 4 to 6 of the spores suspension application. The overall infected aphids produced fewer offspring on days 4 and 6 when comparison with control. After administration of fungus at concentrations (10^4 and 10^5), activities of (Phenoloxidase, Peroxidase, Acetylcholine esterase and α-Amylase) slightly not significantly increased in aphid with respect to control, but the activity decreased at concentration (10^6). This study declared the enzymatic mechanisms concerned in insect immunity to entomopathogenic fungus and finally lead to death.

**Key words:** Entomopathogenic Fungus, *Beauveria bassiana*, *Aphis craccivora*, Enzymes activities.

**Introduction**

Pest management through biological control is confident using different predators, parasites and pathogens. Entomopathogenic fungi are considered unique compared to other micropathogens. The wide host range of fungi, way of its pathogenicity and control of juice sucking pests such as mosquitoes, aphids and chewing pests (Fan *et al*., 2007; deFaria and Wraight, 2007). Entomopathogenic fungi can be used for pest control and do not affect on other non-target organisms (Khetan, 2001).

There are over 700 species of entomopathogenic fungi reported (Rabinda and Ramanujam, 2007), however, some species have been effectively utilized widely as microbial control agents such as *Beauveria bassiana*, that attack a wide range of pests. The fungus *B. bassiana* (Hyphocreales, Ascomycota) is a facultative saprophytic fungus infect and kill insects and other arthropods. This fungus inhabit a variety of plant species as endophytes (Barelli, *et al*., 2016). Entomopathogenic fungi infect the insect through penetration of the cuticle. Germ tubes grow through the layers of the cuticle using the enzymatic action and finally enter the haemocoel (Anderson, *et al*., 1995). Aphids are dangerous insect pests all over the world. Asexual reproduction of aphid (parthenogenesis) increase the population mass. It is important to appreciate the effect of entomopathogenic fungi on the aphid pest at different developmental stages (Wang and Knudsen, 1993).

The faba bean, *Vicia faba* is one of the most important leguminous crops as a source of plant protein in Egypt. This crop is highly susceptible to infestation with cowpea aphid, *Aphis craccivora* (Koch) (El-Defrawi, 1998). The cow pea aphid is the most notorious pest causing considerable damage to bean plants. The nymphs and adults suck plant juice and cause serious damage on the seedling and pod bearing stage. The aphid spread plant diseases through the transmission of pathogens and resulting in yield drop (Dixon, 1998). Direct feeding of aphids and its excretion of honeydew cause pathogenic effects and saprophytic fungal development (Klingauf 1987). Some aphids use of symbiotic bacteria such as *Buchnera aphidicola* to synthesis amino acids deficient in their diet.

**Corresponding Author:** Wafaa L. Abdou, Pests & Plant Protection Department, National Research Centre, Dokki-Cairo, Egypt. E-mail: abdouwafaa@rocketmail.com
It is reported that aphids use proteinases in protein digestion (Pyati et al.
2011). Aphids have several enzymatic proteins as phenoloxidases, hydrolases, peroxidases, acetyl choline esterases, glucosidases and esterases (Arimura, et al., 2005). The study of enzymes shows that Aphis fabae have different enzymes their host plant. Some bio-insecticides affect on the activities of transaminase enzymes (GOT &GPT) and carbohydrate hydrolyzing enzymes (invertase and amylase) (Mead, 2000).

The aim of this study shows the ability of entomopathogenic fungus B. bassiana to control aphid species A. craccivora in its adult stage and its effects on mortality and antioxidant enzymes activity of the aphid which could be used to develop new management control strategies.

Materials and Methods

Rearing of the insect:

A stock culture of A. craccivora was maintained on broad bean plant, Vicia faba under laboratory conditions of 20 ± 5ºC and 65 ± 5% R.H. for several generations. In all experiments, the insects were put on fresh broad bean plants cultivated in small pots (8 cm in diameter, one plant/pot) and enclosed individually in bell jar, 10 cm diameter, 22cm long, the tops of which were covered with muslin held in place with rubber bands.

Cultivation of the fungi

B. bassiana, was kindly obtained from University of Florida. (Apopka strain 97) and reproduced in Microbiology Dept., N. R. C. Cairo, Egypt. The fungi were primarily purified using the monospore technique. They were propagated in Petri-dishes (10cm) on potato dextrose agar medium (PDAM) enriched with 1% peptone, 4% glucose, and 0.2% yeast and incubated at 26 ºC. Seven-days old cultures with well developed spores were harvested by washing with 10 cc sterilized water then added 3ml, Tween-80 and completed to 100 ml water and used as stock suspension with known spore concentration then kept in a refrigerator at 4 ºC, from which the fungi were sub-cultured to be used in laboratory evaluation tests (infectivity and bioassay tests) adjusted as conidiophores concentration of 1X10^8/ml. Large amount of conidia spores, if needed, were produced by culturing the fungus on liquid medium in 1L cell culture glass bottles according to (Rombach, et al., 1988).

Bioassay:

1- Indirect treatment of B. bassiana on faba bean Vicia faba Plant:

Faba bean seedlings were raised in small plastic cups of size 8x7.5cm in the laboratory. Fifteen days old seedlings were used for the bioassay studies. Three different spore concentrations, (1x10^8, 1x10^7 1x10^6, spores ml^-1) were prepared for B. bassiana. Each concentration was replicated three times. The respective concentrations of all fungal spore suspensions were sprayed on the seedlings using an atomizer. One day old adult apterous aphids were put on the faba bean seedlings using a camel hairbrush at 5 aphids per seedling. Totally 15 aphids were used for each concentration. Seedlings were kept under bell jar to avoid the escape of aphid population and to maintain the humidity.

2- Direct treatment of B. bassiana on aphid A. craccivora

Aphids were sprayed directly with (1x10^8, 1x10^7 and 1x10^6) spores ml^-1 of fungus, the treatment aphids were transferred to seedling plant (15day old) cups. Also, 15 aphids were used for each concentration. To determine the reproduction of aphids, the number of offspring (neonate aphids) produced every day was recorded.
Biochemical studies:

Sample preparation and enzyme assays.

Adult insects were prepared for analysis by being homogenized in distilled water (20 mg/ml). Homogenates were centrifuged at 8000 r.p.m. for 15 min at 5°C in a refrigerated centrifuge. Supernatant was separated and kept at -20°C till used directly for the determination of the following.

(i) Peroxidase activity was determined according to Vetter et al. (1958).

(ii) Phenoloxidase activity was determined according to a modification of Ishaaya (1971).

(iii) Ach-E (acetyl cholinesterase) activity was measured according to the method described by Simpson et al. (1964).

(iv) α-Amylase activity was described by Bandani et al. (2009).

Results and Discussion

The toxic effect of Beauveria bassiana on aphids A. craccivora using direct method of application.

Table (1) shows the latent effect of fungus spores suspension on the aphid adults after inoculation with different fungi spores (10^2, 10^4 and 10^8 spores/ml). It was found that the mean adult mortality percentages were increased with the increasing of both the time and spores concentrations. All the three concentrations increased the mortality percentages of aphids, the mortality of aphids after 2, 4 and 6 days of direct treatment were recorded as 33.33, 80.00 and 60.00 for concentration one; 6.67, 26.67 and 60.00 for concentration two and 0.00, 26.67 and 0.00 for concentration three, respectively. It is clear from the results of indirect treatments that there was increasing in mortality percentage from day 4 to 6 of the spores suspension application which depicted the lethal effect of the treatments. The mortality percentages were detected (calculated by Abbott’s formula). The obtained results appeared that the fungus induced highly mortality against faba bean aphids. These results are in agreement with (Verma et al., 2007) who reported that, Trichoderma spp. have been widely used as antagonistic fungal agents against several pests. Also, (Van et al., 2007) showed that twelve strains of different entomopathogenic fungi were screened for aphid control. They found that Metarhizium anisopliae and B. bassiana were the highest virulent pathogenicity for both Myzus persicae and A. gossypii. The effects of the entomopathogenic fungus, Trichoderma hamatum on the adult cotton aphid A. gossypii declared that the LC50 and LC90 were 35.778 and 361.799 spores/ml respectively against the adult aphid. The mortality percentages were 20.88; 26.39; 31.28 and 65.15% at 10^2; 10^3; 10^4 and 10^8 spores/ml of T. hamatum, respectively (Khaleil et al., 2016).

Table 1: Mortality % of adult aphids Aphis craccivora after direct and indirect treatments with (Beauveria bassiana).

<table>
<thead>
<tr>
<th>Fungal conc.</th>
<th>Corrected mortality* of adults % at intervals</th>
<th>Average %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Two days</td>
<td>Four days</td>
</tr>
<tr>
<td>10^8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10^4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10^2</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

* Percentage of Corrected mortality was calculated according to Abbott’s formula.

D) Direct treatment. (Ind) Indirect treatment

The toxic effect of fungus on offspring production A. craccivora using direct and indirect method of application.

All the three concentrations of B. bassiana reduced significantly the reproduction of aphids (Neonate aphids). The average number of neonate aphids after direct treatment were recorded as 12.67 ± 8.08 for concentration one, 48.33 ± 27.02 for concentration two and 38.67 ± 32.39 for concentration three respectively, when compared to control (84.33 ± 18.56) (see Table 2). The neonate
aphids mortality are directly proportional with to the concentrations of fungus. The response was dose-dependent (i.e. the highest concentration resulted in 84.98% mortality), while the lowest concentration was 54.15%.

Table 2: The effect of direct treatment of Beauveria bassiana on A. craccivora reproduction.

<table>
<thead>
<tr>
<th>Fungal conc.</th>
<th>No. of live Neonate aphids</th>
<th>Average</th>
<th>F-ratio</th>
<th>% of Mort.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Two days</td>
<td>Four days</td>
<td>Six days</td>
<td></td>
</tr>
<tr>
<td>$10^3$</td>
<td>22</td>
<td>8</td>
<td>8</td>
<td>12.67±8.08*</td>
</tr>
<tr>
<td>$10^4$</td>
<td>76</td>
<td>22</td>
<td>47</td>
<td>48.33±27.02m</td>
</tr>
<tr>
<td>$10^5$</td>
<td>76</td>
<td>18</td>
<td>22</td>
<td>38.67±32.39m</td>
</tr>
<tr>
<td>Control</td>
<td>102</td>
<td>86</td>
<td>65</td>
<td>84.33±18.56</td>
</tr>
</tbody>
</table>

*Significant of differences from “un-treated” controls according to Duncan at P < 0.05.
Ns denotes not significant

Table 3 shows that treated aphids with indirect application, the neonate of aphids found with the highest concentration ($10^3$) of fungus a significance decrease of the average number neonate 39±19. But the 2nd and 3rd concentrations are not significant decrease on offspring number with respect to control (84.33±18.56). Mortality ranged from 37.5 to 53.75% among the fungus concentrations. The overall infected aphids produced fewer offspring on days 4 and 6 when compared with control.

Our results are similar to (Kim 2007) who observed that the total fecundity of aphid A. gossypii was 41 ± 7.3, 26 ± 0.8 and 22 ± 5.7 nymphs per female at $10^3$, $10^4$ and $10^5$ conidia/ml, respectively compared with 51 ± 2.0 nymphs per control female. The period of reproduction was significantly reduced with increasing spore concentration. The fecundity of pea aphids, A. pisum, infected with B. bassiana and P. neoaphid, reduced within 24 h of infection (Baverstock et al., 2006).

Table 3: The effect of indirect treatment of Beauveria bassiana on A. craccivora Koch reproduction.

<table>
<thead>
<tr>
<th>Fungal conc.</th>
<th>No. of live Neonate aphids</th>
<th>Average No. of live Neonate</th>
<th>F-ratio</th>
<th>% of Mort.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Two days</td>
<td>Four days</td>
<td>Six days</td>
<td></td>
</tr>
<tr>
<td>$10^3$</td>
<td>60</td>
<td>23</td>
<td>34</td>
<td>39±19</td>
</tr>
<tr>
<td>$10^4$</td>
<td>68</td>
<td>50</td>
<td>4</td>
<td>50.67±17.01m</td>
</tr>
<tr>
<td>$10^5$</td>
<td>82</td>
<td>50</td>
<td>26</td>
<td>52.67±28.09m</td>
</tr>
<tr>
<td>Control</td>
<td>102</td>
<td>86</td>
<td>65</td>
<td>84.33±18.56</td>
</tr>
</tbody>
</table>

*Significant of differences from “un-treated” controls according to Duncan at P < 0.05.
Ns denotes not significant

Effects of Beauveria bassiana on enzyme activities in A. craccivora.

Study of enzymatic activities in the A. craccivora in response to fungal infection Phenoloxidase. (PO)

Insect phenoloxidase (PO) is an important enzyme in defense reaction against pathogens and parasites. This enzyme present in the hemolymph of the majority of insects as an inactive proenzyme, called prophenoloxididas. The activities of phenoloxidase in A. craccivora after fungal treatment was assessed, as shown in table (4) there were alterations in PO level with different concentrations.

These results showed that the activities of PO in A. craccivora were not significantly affected by fungus. The slightly increased in enzyme activity was recorded at concentration one (0.093±0.013) than in concentration two and three (0.054±0.025, 0.048±0.007) respectively compared with control (0.034±0.010). This enzyme replaces phenols to quinones, which polymerise to form melanin, which acts against pathogens and parasites (Söderhäll and Cerenius 1998), (Cerenius and Söderhäll 2004). Interestingly, high levels of PO in insects lead to increase resistance to pesticides (Shouzhu et al., 2009). Our results on PO activities are in agreement with those reported by (Shereen et al 2012) who found that this enzyme fluctuated between decreasing and increasing at different doses. Temporary increase in phenoloxidase activity occurred in the haemolymph in both 5th instar nymphs and adult locusts after injection of blastospores from Met 189 (Mullen and Goldsworthy 2006). Treated nymphs of Locusta migratoria with Metarhizium anisopliae, showed high level in PO enzyme during the early period but decreased during the later period (Jia, et al., 2016).

570
Peroxidase (POD)

Enzymes analysis revealed that there were not significant differences (P ≤ 0.05) in Peroxidase activity of *A. craccivora* at the three tested concentrations. Slightly increase in peroxidase activity was recorded on highest concentration (0.122±0.005). Peroxidase level shows decrease at concentration (10³) (0.074±0.036) higher than that at concentration (10⁴) in comparison with (0.067±0.014) in the control (see Table 4).

Antioxidant enzymes such as superoxide dismutase (SOD), and peroxidase (POD) provide defenses against pathogens and insecticides (Felton, *et al.*,1995).Our results are consistent with those obtained by (Jia, *et al.*, 2016) who found that when *L. migratoria* were treated with *M. anisopliae* SOD and POD activities increased during the early period of infection.

**Acetylcholinesterase (AChE)**

This enzyme plays an essential role in the breakdown of acetylcholine that function as neurotransmitters of most animals and insects. It is found in chemical synapses where its activity serves to terminate synaptic transmission. Activities of acetylcholinesterase (AChE) of *A. craccivora* were not significantly different among the three concentrations, with the first concentration having the highest (1.161±0.137), followed by the 2nd (1.019±0.049), and the concentrations three was the lowest (0.584±0.116) with respect to the control (1.035±0.571) (Table 4).

The present findings are in agreement with (Ali, *et al.*, 2017) who showed that (AChE) activity decreased in *Bemisia tabaci* when treated with the fungus *Leccanicillium muscarum*. Also, the activity of AChE in *L. migratoria* under different treatment conditions with the fungus *M. anisopliae* increased during the early period but decreased during the later period (Jia, *et al.*, 2016).

**Table 4**: Biochemical and enzymatic changes in *A. craccivora* treated with fungus as direct spray treatment.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control Means±SE</th>
<th>Spray Treatment</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10⁰</td>
<td>10¹</td>
<td>10²</td>
</tr>
<tr>
<td>Phenoloxidase (Total) (units/mg)</td>
<td>0.034±0.010</td>
<td>0.093±0.013</td>
<td>0.054±0.025</td>
</tr>
<tr>
<td>Peroxidase (Total) (units/mg)</td>
<td>0.067±0.014</td>
<td>0.122±0.005</td>
<td>0.105±0.030</td>
</tr>
<tr>
<td>AcetylCholine esterase</td>
<td>1.035±0.571</td>
<td>1.161±0.137</td>
<td>1.019±0.049</td>
</tr>
<tr>
<td>α Amylase activity</td>
<td>11.560±6.480</td>
<td>33.760±10.21</td>
<td>30.170±1.57</td>
</tr>
</tbody>
</table>

*Significant of differences from “un-treated” controls according to Duncan at P < 0.05.*

Ns denotes not significant.

α-Amylase activity.

Our studies declare that α-amylase enzyme is present in the adults of *A. craccivora*. As seen in Table (4), the activity of α-amylase was 33.760±10.21, 30.170±1.57 and 10.563±5.151 for the tested concentrations in comparison with the control ones 11.560±6.480. Fungus had no significant (F-value = 3.379) effect on the activity of α-amylase in all tested concentrations. The activity of amylase enzyme in adult aphid treated with *B. bassiana* was not significantly increased than untreated aphids during different tested concentration.

Generally, the irregular effects of *B. bassiana* on biochemical activities which ranged between decrease or increase during the tested time intervals was observed in (Table 4).

These results are in harmony with (Abdel-Fattah *et al.*, 1986) who showed that the activities of α-amylase enzyme was much higher at the initial time interval (Zero-time) than at the last one (96 hr.) at the three concentrations used of diflubenzuron and triflumuron (LC15, LC30 and LC50).

Carbohydrates are contributed to the structures and function of insect tissues. Metabolism of carbohydrates controlled mainly by amylase, trehalase and invertase enzymes, which play role in the digestion and utilization of carbohydrates by insect (Wigglesworth, 1972). (Khaleil, *et al.*, 2016) found that the carbohydrates hydrolyzing enzymes; amylase, trehalase and invertase of the cotton...
aphids were increased after 96 hrs post-treatment of Entomopathogenic Fungus (T. hamatum) in 31.17, 39.24 and 24.28%, respectively as compared to control.

**Acknowledgement**

Many thanks to Prof. Dr. Magda M. Sabbour, National Research Centre, Egypt for providing the entomopathogenic fungus.

**References**


