Effect of UV-C Rays in Entomopathogenic Fungi *Beauveria bassiana* (Bals.) to Control Apple Fruit Moth *Cydia pomonella* L. (Tortricidae: Lepidoptera)

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https://doi.org/10.59658/jkas.v10i3.1237

Received:
June 6, 2023

Accepted:
July 12, 2023

Published:
Sept. 10, 2023

Abstract

The study was conducted to investigate the behaviour and development of the entomopathogenic fungus *Beauveria bassiana* exposed to UV-C rays and exposure periods under laboratory conditions in pupae of the apple moth *Cydia pomonella* L. The results indicated that fungus exposed to UV-C rays for 5 minutes became more effective in controlling moths, compared with concentrations (2.25×10^4, 10^5, 10^6 and 10^7 spore/ml) of fungus exposed to UV-C rays for 10 minutes. The results also showed that pupae and insects emerging from them at the age of (1-2) days were more sensitive to fungus concentrations than pupae at the age of (10-12). As the percentage of emergence decreased, with the increase in fungus concentrations. Furthermore, the levels of proteins in the blood for insects produced from pupae aged (1-2) and (10-12) days decreased to 8.30 and 16.10 µg/ml ,respectively, when were exposed to the irradiated fungus *B. bassiana* for 5 minutes at a concentration of $2.25x10^7$ spores/ml. Compared to insects produced from pupae exposed to non-irradiated pathogenic fungi, that was 20.12 and 25.15 µg/ml, respectively, and in comparison with insects produced from pupae exposed to the fungus for 10 minutes at the age of (1-2) and (10-12) days, respectively, as it reached 12.50 and 26.10 µg/ml and for the same concentration. In addition to a decrease in the levels of sugars in the blood, reaching 20.00 and 24.00 ml/ µg in insects produced from pupae aged (1-2) and (10-12) days, respectively, exposed to the irradiated fungus for 5 minutes and with the same concentration. The percentages of blood components decreased quantitatively and qualitatively, as the number of hemocytes for insects that emerged from pupae exposed to the irradiated fungus for 5minutes reached 70 and 95 cells/ml, respectively, compared to 92 and 110 cells/ml for insects that emerged from pupae exposed to the irradiated fungus for 10minutes and with the same concentration.

Keywords: Codling moth, biological control, Entomopathogenic fungi

Introduction

Insect pests are one of the main reasons for the decline in the value and quality of agricultural and animal production, as they are an obstacle to the trade of fruits and vegetables. It also leads to severe damage to the weight, quality and economic value due to the spoilage of quite a few of these products. *Malus domestica* apple is one of the most important fruits with important nutritional and economic value. Its fruits are rich in carbohydrates, protein, fatty substances, organic acids and mineral salts. Apple trees are infected with some important insect pests that cause great economic damage and cause a significant decrease in yield [1]. The apple moth, *Cydia pomonella* (L.), is the main pest of apple orchards. Apples are the insect's favorite host, but they can infect walnuts, pears, quinces, apricots, peaches, almonds, and cherries [2], as the larvae cannot feed on leaves, as they depend mainly on fruits as a source of food. What stimulates the insect to lay eggs is the smell of the fruits, which is attractive to the newly emerged larvae to enter the fruit and cause damage [3]. This pest is fought by using chemical pesticides, as a wide spectrum of them is used frequently and randomly in most cases, and this leads to an increase in production costs and a decrease in the value of the economic return for the farmer, and pesticides kill vital enemies in the environment. On the other hand, the apple fruit moth showed resistance to many Insecticides used [4]. Entomopathogenic fungi spread within a wide range of ecosystems, and infection occurs in these insects, the most important of which is the fungus *Beauveria bassiana* (Bals.). *B. bassiana* the successes achieved by pest-pathogenic fungi in limiting the spread of some incoming pests without affecting the environment constituted an essential element in the control of agricultural pests and a fertile ground for scientific research. It is found in the natural environment and is considered within the integrated management programs as an alternative to chemical pesticides that caused an imbalance in the biological balance and the emergence of resistance in many insect pests [5]indicated that the fungus *B. bassiana* caused a high mortality rate in the larvae of the apple fruit insect *C. pomonella*, especially the larvae of the first instar, after 8-10 days of treatment. One of the physical methods that were commonly used during the last decades of the last century and the current one in controlling storage insects is the use of electromagnetic energy such as infrared, gamma rays, and ultraviolet rays [6].

Materials and Methods

Insect breeding

To conduct experiments, a laboratory colony of apple fruit moth *C. pomonella* was established inside a sterile glass vial with a diameter of 14 cm and a height of 22cm, and then 10 pairs of newly emerged insects, aged 24-48 hours, were released into it. Adult insects were fed on cotton swabs saturated with a 10% sugar solution, replaced daily, inside a petri dish. 250 g of apples were placed inside each glass bottle to feed

the larvae; cotton swabs were also placed for the purpose of hindering the larvae. The mouth of the bottle was covered with a damp cloth and fixed with a rubber band to prevent the exit of insects. Then it was placed in the incubator at a temperature of 25 ± 2 °C and a relative humidity of 60 ±5% with a lighting period of (8 dark: 16light) hours for 25days. They were monitored daily to obtain immature offspring. Breeding continued for two generations before experiments were conducted on them. The experiment included four replications for each treatment, and the models were exposed to four different concentrations of *B. bassiana* fungus exposed to ultraviolet radiation with a wavelength of 312nm. The mortality rate and the corrected mortality rate were calculated by applying the following equations [7].

$$\textbf{Mortality rates} = \frac{\textbf{The numbers of dead insects}}{\textbf{Total numbers of insects}} \textbf{X100}\ \%$$

$$\textbf{Corrected mortality rate} = \frac{\textbf{Percentage of mortality in the treatment} - \textbf{the percentage of mortality in the control}}{\textbf{100} - \textbf{Percentage of mortality in control}} \textbf{X100}\%$$

Then the values were angularly transformed, and the data was analyzed at a probability level of 0.05[8].

Preparation of the basic fungal suspension of *B. bassiana*

A suspension of fungus spores was prepared using a petri dish containing a growing fungus colony, to which 5ml of sterile distilled water was added containing 0.02% Tween-20 solution as a moisture retainer. The spores were mixed; then the contents were filtered using a glass funnel containing two pieces of gauze inside a conical flask with a capacity of 150ml, then 10ml of distilled water was added to it to remove all spores from the sides of the cloth, then the solution was shaken for 15 minutes with the Vortex device. In order to separate the spores from mycelium. After mixing and shaking, distilled water was added to complete the volume to 100 ml, which represents the base suspension[9], and then 0.1ml was taken from the base suspension by a Pasteur dropper and placed on a spore counting slide. When calculating the number of spores at the power 40x in the counting cell, it reached 90 spores. And when multiplied by $2.5x10^5$, which is the conversion factor for the cell, according to the following equation:

$90 \times 2.5x10^5 = 2.25 \times 10^7$ spore /ml. Thus, a basic suspension [10] was obtained, and it is a source for obtaining the required concentrations.

Prepare the required concentrations of *B. bassiana* suspension

To obtain the required concentrations of the original suspension of the *B. bassiana* fungus, it was diluted to reach the required concentration. The quantity is taken from the original suspension (ml) = the required concentration / the original concentration of the fungus. To obtain 20 ml of the original suspension of the fungus at a concentration of 10^6 spore/ml:

10^6 /10^7 = 0.1ml, after which the product is multiplied by the amount of solution to be obtained (20 ml), so that the result becomes 2ml, and thus 2ml of the main suspension is taken, and 18 ml of distilled water containing Tween-20 solution is added to it, and thus we obtained 20ml with a concentration of 10^6 spore/ml. Thus, for the other concentrations (2.25×10^4, 10^5,10^6 and 10^7 spore/ml) prepared for different treatments, the conical flasks (volume of 30ml) were kept in the refrigerator at a temperature of 4C^o until the experiments were carried out.

Distinguish females and males

The females were separated from the males in the last larval age (fifth), as the male larvae were distinguished from the females through a red-purple spot that appears on the fifth abdominal ring from the dorsal side in the last larval age of the males. This spot does not appear on the female larvae [11]. The larvae were examined when they transformed into the cocoon stage or shortly after their transformation, as this difference can be seen with the naked eye and then the males and females' cocoons were separated in special boxes until they emerged.

Collecting Pupae of insect

The pupae were carefully collected from inside the breeding bottles using forceps, and divided into two groups, aged (1-2) and (10-12) days. Four replicates were used for each treatment, and in each replicate were five pupae for each age group in plastic dishes with a diameter of 9cm and a height of 2cm containing filter paper moistened with distilled water to maintain moisture and prevent dehydration. The flasks containing the concentrations of the pathogenic fungus were placed at a distance of 10cm from the radiation source with a wavelength of 312nm. Then they were exposed to UV-C rays for periods of 5 and 10minutes, after which the pupae were sprayed in each replicate and for each age group with the pathogenic fungus according to the required concentrations. The control was exposed to the pathogenic fungus that is not exposed to UV-C rays, and then each replicate was transferred separately into volumetric flasks with a capacity of 250ml containing a petri dish containing a piece of cotton saturated with a 5% sugar solution to feed the emerging insects. The numbers of dead pupae for each replicate and the development of each were recorded. All replicates, and the control treatment were placed in the incubator under the same previous storage conditions to monitor their development and record the results.

Statistical analysis

The results of the current study were analyzed by the method of factorial experiments according to the complete random design (CRD). The statistical program GenStat2012 was used to analyze the results. The significant differences between the treatments were compared with the Least Significant Difference L.S.D test. At a probability level of $P<0.05$[8].

Results and Discussion

The results shown in Tables (1 and 2) indicated an increase in the mortality rates of pupae and, consequently, a decrease in the number of emerging insects. The concentration of the insect-pathogenic fungus *B. bassiana* exposed to UV-C at a wavelength of 312nm for 5 minutes increased, compared with the concentrations of the fungus exposed to UV radiation. UV-C rays for 10 minutes increased the rates of deformities in the emerging insects. A weakness was observed in the movement of the resulting insects due to the emergence of fungal filaments from the bases of the wings. Then after several days of exposure to fungus spores at high concentrations, symptoms appeared on the infected insects, as they were characterized by a lack of movement. All the tested concentrations showed mortality rates in the insects emerging from the treated pupae in varying proportions. The mortality of newly emerged insects can be attributed to mycotoxins secreted by pathogenic fungi, as experiments have shown that pathogenic fungi produce many toxic compounds for insects that affect the metabolic processes of the insect[12]. It is possible that the insects died because of mycotoxins and not because of fungus growth and spread among infected insects.

The results of the current study indicated a high percentage of mortality in pupae at the age of (1-2) and (10-12) days, with an increase in the percentage of concentrations of the pathogenic insect fungus of *B. bassiana*, as the highest percentage of mortality reached 82.30 and 35.33% at a concentration of 2.25×10^7 spore/ml for pupae, aged (1-2) days, with an exposure period of 5 and 10 minutes, respectively. As for the mortality rates of pupae at the age of (10-12) days and with the same concentration and time periods, they decreased as they were 52.30 and 22.10% ,respectively (Tables 1 and 2). This is the result of the invasion of fungal hyphae and toxins secreted by fungus pathogenic to insects, as the results of [13] that the indicated the fungal cells grow and develop inside the body of the infected insect after penetrating the body wall. The growth of the fungus within the bodily fluids of the insect leads to the weakening of the immune system. Then the invasion of all internal tissues. [14] indicated that the effect of the fungus at all concentrations extended to insects resulting from pupae that did not die. The results showed a decrease in the levels of sugars in the blood with the increase in the levels of fungus concentration, and the decrease was greater in the treated pupae at the age of (1-2) days compared to the treated pupae at the age of (10-12) days.

Table (1): The effect of the *B. bassiana* exposed to UV-C radiation for 5 minutes on pupae aged (1-2) and (10-12) days and its development of the apple fruit moth *C. pomonella*

Age (10-12) days				Age (1-2) days				Concentration spore /ml
No. of hmocytes /ml	The level of sugars in the blood µg/ ml	Ratio of proteins in the blood µg / ml	mortality ratio %	No. of hemocytes /ml	The level of sugars in the blood µg/ ml	Ratio of proteins in the blood µg/ml	mortality ratio %	

130	45.10	25.15	26.00	135	42.33	20.12	32.10	Control ×2.25 10^6
112	42.00	20.00	35.22	110	35.20	15.12	45.05	× 2.25 10^4
110	40.10	20.30	42.33	98	30.15	15.10	52.50	× 2.25 10^5
94	35.10	18.10	50.10	102	25.10	12.00	70.20	× 2.25 10^6
95	24.00	16.10	52.30	70	20.10	8.30	82.20	× 2.25 10^7
0.48	0.85	0.88	9.00	8.20	11.60	5.19	5.98	L.S.D.

As it reached 20.10 and 24.00 µg/ml for the insects emerging from the pupae at the age of (1-2) and (10-12) days, respectively (Table1), which were exposed to the fungus at a concentration of 2.25×10^7 spores/ml for 5minutes compared to 10.20 and 40.00 µg/ml, respectively (Table 2) for insects emerging from treated pupae at the same concentration and for the same age group for 10minutes. As well as the levels of protein in the blood of emerging insects of the same age groups and the concentration, it decreased to 8.30 and 12.50 µg/ml for pupae aged (1-2) days at a concentration of $2.25x10^7$ spores /ml for an exposure period of 5 and 10minutes, respectively, compared to 16.10 and 26.10 µg/ml for a period of exposure of 5 and 10 minutes, respectively, to insects that emerged from pupae at the age of (10-12) days and the same concentration. The ability of spores to germinate and penetrate host cuticles can be inhibited by lack of moisture required for spore germination, failure to utilize available nutrients on the host surface, or absence of certain factors necessary to identify suitable hosts or potential penetration sites[15]. As for the percentages of blood components of hemocytes in the insects emerging from the treated pupae, they also decreased with the increase in the concentration of the fungal solution. It reached 70 and 95 cells/ml for insects emerging from pupae treated with the concentration of $2.25x10^7$ spores/ml at ages (1-2) and (10-12) days ,respectively, for a period of 5 minutes (Table 1), and it reached 92 and 110 cells/ml. of insects emerging from pupae treated with the same concentration at the age of (1-2) and (10-12) days, respectively, for a period of 10minutes (Table 2), as indicated[16] that UV-C rays works to cause chromosomal changes in the cell and thus leads to the occurrence of genetic mutations in pathogenic fungi. It was observed that there was a decrease in the size of the ovaries of female apple moths emerging from pupae treated with a concentration of $2.25x10^7$ spore /ml at the age of (1-2) and (10-12) days, respectively, for a period of 5 minutes, as it reached 4 mm compared to 7mm in control group. The results of the current study also showed that the mortality rates in all concentrations did not reach 100% except for the concentration 2.25×10^7 spore/ml treated with UV-C rays for 5 minutes, as the highest rate was 82.20% for pupae aged (1-2) days after 10 days. of the treatment (Table1).

Table (2): The effect of the *B. bassiana* exposed to UV-C rays for 10 minutes on pupae at the age of (1-2) and (10-12) days and its development of the apple fruit moth *C. pomonella*

Age (10-12)days				Age (1-2)days				concentration spore /ml
No. of hemocytes /ml	The level of sugars in the blood µg /ml	Ratio of proteins in the blood µg/ml	mortality ratio %	No. of hemocytes /ml	The level of sugars in the blood µg/ml	Ratio of proteins in the blood µg/ml	mortality ratio %	
142	52.00	32.90	22.30	140	40.10	26.15	38.00	×2.25 control 10^6
150	46.50	32.10	18.10	138	36.20	18.30	15.30	$10^4 \times 2.25$
143	45.20	30.20	22.10	130	30.33	16.20	30.10	$10^5 \times 2.25$
125	40.10	28.00	25.00	115	28.15	12.20	32.00	$10^6 \times 2.25$
110	40.00	26.10	22.10	92	20.10	12.50	35.33	$10^7 \times 2.25$
3.69	5.60	0.51	12.47	6.41	1.03	12.10	13.34	L.S.D.

The reason may be attributed to the fact that the insect's immune system can defend against fungal invasion at low concentrations, and its ability decreases at high concentrations. One of the familiar phenomena in insects when infected with spores of pathogenic fungi is the emergence of activity of many blood cells to surround these spores and pieces of hyphae that invade the body of the insect, which is known as phagocytosis, as plasma blood cells are attracted to the fungus and devour or encapsulate it and form granular bodies within which cells may dissolve invasive fungi [17]. The results of the current study (Tables 1 and 2) showed that pupae and insects emerging from them at the age of (1-2)days were more sensitive to fungus concentrations than pupae at the age of (10-12)days, as mortality rates increased, protein and sugar levels decreased, and the number of hemocytes in the blood increased. Perhaps the reason for this is that the cuticle layers that envelop the pupae at the age of (1-2) days are thinner and more flexible than the pupae at the age of (10-12)days, which affects them faster and is more easily penetrated by the fungal hyphae compared to the older pupae. The reason may be due to the structures that make up the wall of the insect's body, such as the presence of the waxy substance, in addition to other factors that depend on the type of host insect and the appropriate temperatures and humidity for the growth of the fungus, which in turn prevents the arrival or germination of fungal spores except in the case of high concentrations of the fungus. Environmental conditions, especially

temperature, humidity and solar radiation, have an important role in the ability of pathogenic fungi to infect and produce spores.

The current study indicated that exposure of concentrations of the entomopathogenic fungus *B. bassiana* to UV-C rays with a wavelength of 312nm for 5minutes becomes more effective in controlling apple fruit moth *C. pomonella*, compared with concentrations of fungus exposed to UV-C rays for 10minutes. The study's results also showed that the pupae and insects emerging from them at the age of (1-2)days were more sensitive to *B. bassiana* fungus concentrations than pupae at the age of(10-12).

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