Research Article

# Test of vitality of fungus Beauveria bassiana (Bals.) Vuill. on eggs and larvae of moth Figs Ephestia cautella (Walk.) (Lepidoptera: Pyralidae)

**Maan Alsalihi**
[1]biology department, college of science, Mustansiriya University, IRAQ
*Correspondent Author Email: maanalsalihi@uomustansiriyah.edu.iq

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## Abstract

The results showed the effect of different concentrations (1x $10^4$, 1x $10^6$, 1x $10^8$) Spore / ml aqueous suspension of commercial isolation of the fungus *Beauveria bassiana* (Bals.) Vuill. on eggs and second and fourth larval instars of the moth figs *Ephestia cautella* (Walk.) Under laboratory conditions. The lowest hatching rate at 1 x $10^8$ spore / ml was 15.3% after 5 days of treatment and was 60.7% at a concentration of 1 x $10^4$ spore / ml. The rest of the larvae were followed until they reached the stage of the pupa and the adult. The mortality for the second larval instar were highest at 1 x $10^8$ spore / ml after 7 days of treatment reaching 85.22%, The lowest rate is 5.6% after 2 days of treatment at 1 x $10^4$ spore / ml concentration. The concentration of 1x $10^8$ spore / ml had the highest effect after four days of treatment on the fourth larval instar the mortality was 89.6%. The lowest percentage pupation was 52.7%, and the lowest adults emerging were 50.2%.

**Keywords:** Smart Surveillance System, Anomaly Events, Abnormal Behavior, Anomaly Detection, Abnormal Detection.

## الخلاصة

أظهرت النتائج تأثير التراكيز المختلفة (1 × $10^4$، 1 × $10^6$، 1 × $10^8$) سبور / مل معلق مائي للعزلة التجارية للفطر *Beauveria bassiana* (Bals.) Vuill. على البيض والعمر الثاني والرابع ليرقات عثة التين *Ephestia cautella* (Walk.) المعاملة، وبلغ 60.7٪ عند تركيز 1 × $10^4$ بوغ / مل. وتم مراقبة بقية اليرقات حتى وصلت إلى طور العذراء والبالغات. أعلى نسبة قتل للعمر اليرقي الثاني بلغت 85.22% عند تركيز 1 × $10^8$ بوغ / مل بعد 7 أيام من المعاملة، وأدنى نسبة قتل بلغت 5.6٪ بعد 2 يوم عند تركيز 1 × $10^4$ بوغ / مل. وعند تركيز 1 × $10^8$ بوغ / مل أعطى أعلى تأثير بعد أربعة أيام من المعاملة على العمر اليرقي الرابع بلغت نسبة القتل 89.6٪. أما أقل نسبة للتعذر فقد بلغت 52.7٪، بينما أقل نسبة لبزوغ البالغات كانت 50.2٪.

## Introduction

The moth figs (moth dates) *Ephestia cautella* (Walk) was first recorded in Iraq by [1]. Noted that the moth dates larvae infested fall dates from the Palm and the dates lagging behind the harvest, and begin to infested the orchard and continue in the pistons and stores throughout the year. Explain [2] the presence of two main factors that make this pest dangerous to the dates stored and the emergence of resistance in the insect against chemical pesticides and the nature of this type of larvae in terms of feeding within dates. Pointed out the moth figs was the major pest of dried fruits and plays a key role in global trade [3]. The common control method for this pest is the use of $CH_3$ Br, which has gradually begun to recede to increase its toxicity and replace it with safer alternative materials and methods. Indicated that the genus *Ephestia spp*. [4].

One of the most important pest in flour mills in countries with hot climates where the damage caused severe in all winter products. Several chemicals have been used to control the moth figs pest, the most important of which is

methyl bromide, the only material approved in the sterilization dates commercially in Iraq since 1953, which has been replaced with Fustoxine. Over the past two decades, the world has seen growing interest in biotic factors as a means of controlling economically destructive insect pests to rationalize the use of chemical pesticides that have become a threat to human health and the environment, as well as the development of resistance to many insect pests of such chemical pesticides as a result of random and repeated use.

Biological control involves the use of an organism, whether predator, parasitoid, or pathogen, for the purpose of controlling or destroying another living organism. Many species of organisms that are considered natural enemies of harmful insects reduce or reduce the number of certain insect pests naturally. Fungus *Beauveria bassiana* (Bals.) Vuill belongs to the class Deuteromycetes (Hyphomycetes) which is where mycelium divided and sexual phase is unknown to date and the fungus is propagated non-sexually through the formation of spores [5], and it affects a large number of economic insects, causing Disease White muscardin [6]. However, the common method of attacking the host by this fungus is through the body wall [7]. *B. bassiana* produces a variety of toxins including Beauvericin and Bassianin, Bassianolide, Beauverolides, and Tennellin. These toxins play a role in killing the host by dissolving the tissues, degradation the cells, and re-germinating exiting the host body, forming the conidia and restoring the life cycle of the fungus [8]. Studies have shown that fungus does not produce plant-damaging substances and does not have a negative effect on the environment, nor does it have a parasitic ability on humans and animals, which suggests it to be an effective factor in control programs [9]. The fungus was tested against three Lepidoptera larvae *Pieris rapae* (L.) and the diamond-back moth *Plutella xylostella* (L.) and the *Trichoplusia ni* (Hubner) worm studied the sensitivity of three Lepidoptera larvae to Boverin extract from *B. bassiana*, and found that 5% of the fungicide, equivalent to 7.4 x $10^{14}$ spore / ml, reduced the field density of the *Trichoplusia ni* insect to 50% and reduced the damage to leaves to 87% [10]. In a study of the small grain borer *Rhizopertha dominic* on the Amber rice seed stored, the results showed that there were effective killing insect larvae through dipping seeds in suspension microbial bacteria *Bacillus thuringiensis*, and two fungi *B. bassiana* and *Trichoderma Harizanum*, the concentration of 3 x $10^6$ spore / ml of B. bassiana was the best in producing high mortality for insect stages, as it reached 95.0, 88.1 and 95.2% during the one-week period of treatment with both bacteria and fungi, respectively.

## Materials and Methodology

1- **Fungus *Beauveria bassiana:***

Used local isolation of the fungus *B. bassiana* produced in the Agricultural Research center / Ministry of Science and Technology, a concentration was 1 x $10^{10}$ spore / ml and were prepared concentrations of 1 x $10^8$ and 1 x $10^6$ and 1 x $10^4$ spore / ml.

**2-The source of the insect and the method of its breeding:**

This study was carried out in the Laboratory of Insect / Animal House / Department of Biology / College of Science / University of Mustansirhiya, on the insect moth Figs *E. cautella* available in the laboratories of the Department of insects of the Ministry of Science and Technology and reared on artificial food of mixing 81% Crushed wheat, %12Glycerin, 6% molasses, 1% dry yeast to feed larvae, 5% water and honey solution for adult nutrition. Place 250 grams of artificial food in a sterile 11 cm diameter plastic container, 12 cm high, and then release 20 pairs of insect larvae, aged 24-48 hours. Covered with a plastic lid in the middle of it 2 cm diameter hole for ventilation is covered with Mosleen cloth to prevent the insect from escaping. Placed inside the incubator at a temperature of 25 $\pm$2 ° C and relative humidity 65 $\pm$ 5% and photoperiodic light: darkness of 8:16 hours for 25 days, the larvae had reached the larval fifth instar was observed roaming on the walls of the bottle in search of a place where it is pupation. The larvae were collected

at this stage and transferred to sterilize glass bottles containing cotton that was fluffy to pupation the larvae and to get adult insects later with continued five-generation breeding to eliminate chemical residues and other biochemical agents. This colony was used as a source for the eggs on which the treatments was conducted directly or which was the source of the second and fourth larvae instars that were subsequently treated.

**3- The Treatments:**

**Treatment of eggs:**

The eggs were collected by isolated adult newly emerging and placed in lantern bottles high-end covered with a piece of muslin cloth with a hole in the middle a piece of cotton dampened with water and honey to fed the adults. The bottles were placed on a glass petri dish without a lid and placed between the bottom of the lantern bottle and the petri dish black filter paper. So that, the resulting egg falls on it and facilitates its vision and calculation. Prepared four groups of eggs each group representing one treatment and each group consisting of three replicates each containing an average of 20 eggs Each group was treated with a hand sprayer for each concentration of the aqueous suspension of spores of fungus *B. bassiana* and used the following concentrations: 1 x $10^4$, 1x $10^6$ and 1x $10^8$ spore / ml either control treatment was sprayed with water only. Leaving the eggs was treated for half an hour on filter paper to absorb excess water and then transferred to the Petri dishes with a diameter of five centimeters in the bottom of a moisturizing filter paper. Left in the incubator at 25 $\pm$ 2 ° C and relative humidity 65 $\pm$ 5% were observed daily for the purpose of recording hatching rate.

**Treatment of larvae:**

**Second larval instar:**

Used glass Petri dishes diameter 5-cm containing 5 g of industrial food medium. The experiment was carried out by dividing the second larvae instar into four groups, each group representing one treatment with three replicates per treatment, each petri dish containing 10 larvae. Each dish was treated with 1 mL of different treatments. The control was only sprayed with water. Leave the dishes exposed to the air for two hours to evaporate the excess water, closed the dishes tightly after which placed the dishes in the incubator with the same previous temperature and humidity.

**Fourth larval instar:**

Fourth larval instar were collected from the experimental colony and distributed to four groups, each group representing one treatment with three replicates per treatment each petri dish contains 10 larvae and treated as treated larvae second instar. The larvae were examined daily to study the development of different stages, nutrition efficiency, mortality, and monitoring continued until all individuals died or reached the pupa and the adult stage. It calculated the percentage of pupation and the proportion of the emergence of adult from larvae treatment.

**Statistical analysis:**

Experiments were designed according to the complete randomize design (C.R.D.) the least significant difference (L.S.D) was used below a significant level of 0.05 to test the significance of the results. The data were analyzed statistically using analysis of variance table (ANOVA) was used for this purpose statistical program S.A.S [11]. The corrected percentages were converted to angular values for inclusion in the statistical analysis. The mortality was corrected according to the following equation [12]:

$$(\%\ corrected\ for\ mortality) = \frac{(\%\ killed\ in\ trearment) - (\%\ killed\ in\ control)}{100\ - (\%\ killed\ in\ control)} \times 100$$

## Results and Discussion

**Treatment of eggs:** Results of Table 1 showed that there was an effect of the concentrations of *Beauveria bassiana* (Bals.) Vuill on the percentage of hatching of eggs after five days

of treatment, the highest percentage hatching was 60.7% at the concentration of 1 x $10^4$ spore / ml while the lowest percentage hatching was 15.3% at the concentration of 1 x $10^8$ spore / ml. The reason for the low percentage of hatchling to parasitism fungus spores on eggs moth Figs by penetrating the spores' fungus The egg wall and then the germination of spores under the optimal temperature of laboratory conditions, 25 $\pm$2 ° C and relative humidity 65$\pm$ 5% and growth mycelium into the egg, resulting in the failure of growth and development of the embryo within the egg. The results obtained by the fungus were somewhat similar to those obtained on white fly eggs, which studied the parasitic capacity of 6 isolates of *B. bassiana*, the percentage of parasitism ranging from 53.1 - 84.4% after seven days of treatment with aqueous suspension of fungus spores Isolates. The results were less similar to those in a laboratory study in which two isolates of *B. bassiana* were used in parasitism on the eggs of the corn stem borer *Sesamia cretica*, it was found that the isolates were highly efficient in parasitism on the eggs of the insect and reached 97.5% and 95.8% after three days of treatment, the low effect may be due to the virulence of the different isolates of fungi or insect susceptibility. The mortality of newly larvae are hatching from the treated eggs, which exceeded the egg stage, it was observed that there was a slight effect, with the highest mortality at 1 x $10^8$ spore / mL was 42.5%, which differed significantly from the control treatment. This was contrary to [13] where it reached the lowest rate of hatching of eggs 29.8% at concentration 1x $10^6$ spores/ ml for *E. cautella*. This converges with explaining that the parasitism capacity of *B. bassiana* is due to its ability to produce the enzyme chitinase, which plays a key role in the process of decomposition of the chitin in the wall so that the fungal mycelium can penetrate the wall of the egg and destroy its internal contents and consumption.

Vuill on the percentage of eggs hatching and on the mortality of first larval instar produced by the eggs treated for the moth figs *Ephestia cautella*.

Table 1: Effect of the concentrations of the fungus *Beauveria bassiana* (Bals.).

| Concentrations Spore/ml | % hatching eggs after 5 days | % mortality of 1st larval instar after 5 days |
|---|---|---|
| 1x$10^4$ | 60.7 | 20.55 |
| 1x$10^6$ | 35.8 | 30.76 |
| 1x$10^8$ | 15.3 | 42.5 |
| L.S.D. 0.05 | 3.62 | 8.32 |

**Treatment of larvae:**

**Second larval instar:**

In Table 2, the results of the statistical analysis showed that there were significant differences in the percentage of mortality the second larval instar of the moth figs, which increases with the duration of exposure to the fungus and an increased the concentration, reaching the highest mortality 85.22% after 7 days of treatment at 1 x $10^8$ spore / ml, the lowest mortality was 5.6% at a concentration of 1 x $10^4$ spore / ml after 2 days of treatment. This is consistent with what was stated on the beetle larvae *Scolytus spp*, as it is mentioned that the contact with the fungi of the body of larvae and parasitism on them leads to paralyzing movement because of the decomposition of the muscles of the body at the sites of the fungus attack and turn the dead larvae into a mummy.

**Fourth larval instar:**

The results of Table 3 showed that the treatment of the 4th larval instar of the moth figs had a very little effect at the beginning, but increased by increasing the exposure period as the fungus achieved the highest mortality after four days of treatment, reaching 89.6% at the concentration of 1 x $10^8$ spore/ml, this indicates that the time factor is important in biocontrol as the period of exposure to *B. bassiana* increases and the concentration increases as the mortality of the insect increase. And this is consistent with the findings of the [14] which is mentioned when studied the effect of fungus *B. bassiana* on the larval beetle roots of alfalfa *Sitona lepidos*, it was less effective on the last stages, It was also observed that there was an effect on the rate of pupation for larvae to treatment was 52.7 % at the highest concentration of 1 x $10^8$ spore / ml and

significantly different from the lowest concentration 1 x $10^4$ spore / ml. Table 3 also shows that the percentage of adults emergence from the treatment of the last larval instar was affected, with the lowest rate emergence reaching 50.2% at the highest concentration 1 x $10^8$ spore / ml, The treatment at 1 x $10^4$ spore / ml was significantly different from the control treatment. It was observed that the effect of *B. bassiana* on the last larval instars was different from the first instars, and this is due to the fact that the infected larvae are a new source of infection of healthy larvae with their continuous movement, or they get the biggest opportunity fungus spores adhesion on their bodies as a result of this movement. This does not agree with what was said, indicating a gradual decrease in the effect of *B. bassiana* on the larval instars of the corn stem borer. As these larvae progressed, the mortality of larvae decreased. This was contrary to [15] where used three isolates of *B. bassiana*, the mortality for fourth larval instar of *E. cautella* were 13.3, 12.1 and 11.6% at concentration 1x $10^4$ spore/ ml, respectively.

Table 2: Effect of the concentrations of the fungu *Beauveria bassiana* (Bals.) Vuill in the mortality the second larval instar of the moth figs *Ephestia cautella* in the laboratory conditions.

| Concentrations spore / ml | % mortality / Days | | % pupation | % emergence adults |
|---|---|---|---|---|
| | 2 | 4 | | |
| $1x10^4$ | 10.6 | 62.5 | 70.2 | 66.3 |
| $1x10^6$ | 15.8 | 83.2 | 61.4 | 59.8 |
| $1x10^8$ | 25.3 | 89.6 | 52.7 | 50.2 |
| L.S.D. 0.05 | 7.6 | 5.7 | 9.8 | 10.66 |

Table 3: Effect of the concentrations of the fungus *Beauveria bassiana* (Bals.) Vuill in the mortality the fourth larval instar of the moth figs *Ephestia cautella* in the laboratory conditions.

| Concentrations | % Mortality / Days | | |
|---|---|---|---|
| spore/ml | 2 | 4 | 7 |
| $1x10^4$ | 5.6 | 10.7 | 63.75 |
| $1x10^6$ | 10.9 | 16.5 | 74.33 |
| $1x10^8$ | 15.4 | 22.76 | 85.22 |
| L.S.D.0.05 | 7.55 | 6.44 | 5.33 |

## Conclusions

This research has now demonstrated the potential for *Beauveria bassiana* in different concentrations on the mortality rate (eggs, second instar larvae and fourth instar larvae), especially concentration (1x $10^8$ spore/ ml) because the best concentration to mortality the different stage of insect *E. cautella*, Effect of fungus on the eggs and second instar larvae better than the effect on fourth instar larvae. This research can be applied in practice in the storage and control of insect moth figs to eliminate the eggs and larvae of the insect and reduce the proportion of the emergence of adults gradually until the eradication of insect completely. Entomopathogenic fungi are generally considered to be safe in terms of low risks as compared to chemical pesticides, using of entomopathogenic fungi in stored is now considered as one of the most promising alternation to residual pesticides and fumigants.

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