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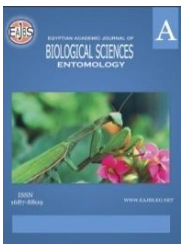

The Effect of the Fungus *Beauviria bassiana* on the Mortality Percentages of the First and Fourth Larval Instar of the Red Flour Beetle, *Tribolium castanium* (Coleoptera: Tenebrionidae)

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ABSTRACT

This study was conducted in the advanced insect laboratory in the Department of Biology / College of Education for Girls / University of Kufa for the academic year 2020-2021 to evaluate the effectiveness of the fungus Beauveria bassiana in mortality percentages of the first and fourth larval instars of the red flour beetle Tribolium castanium. The results of the current study were the increase in the mortality percentages of the first larval instar with the increase in the concentrations of the fungal aqueous suspension, and the highest mortality percentage was 90% obtained on the seventh day of the experiment after being treated with a concentration of (4 %) of the aqueous extract of the fungus, while the highest mortality percentages were 80% in the fourth larval instar, after the 7 days of the experiment and the treatment at a concentration of (4 %).

INTRODUCTION

The red flour beetle, *Tribolium Castanium*, is one of the most important insect pests in Iraq and many regions of the world, as it attacks many food products such as flour, grains, biscuit chips, spices, chocolate, and dried fruits, causing huge economic losses, with a damage percentage 10% - 40% of the crop grains stored in the world. (Sana *et al.*, 2014). The flour beetle is considered one of the most important warehouse insects, due to the presence of grocery stores in homes. Flour infected with this insect has a distinctive smell and moldy taste as a result of the secretion of quinone compounds. Flour infected with this insect also loses many of its properties that make it unsuitable for making bread, such as viscosity and chewiness. To reduce the quantity and quality of food as well as change the chemical composition of infected grain. (Mohamed Waddah, 2012). Chemical pesticides are still one of the most important means of controlling insect pests because of their effectiveness in increasing agricultural production by controlling pests and diseases that affect them, and the use of pesticides has achieved a high degree of protection and prevention for humans against the dangers of some insects and pests that transmit dangerous diseases, but the use The intensive use of different pesticides in plant protection programs leads to an imbalance in the environment in addition to the harmful effect on

non-target organisms, which leads to an imbalance in the environmental balance as a result of the absence of adherence to the scientific and health principles that adhere to this use. Therefore, given the danger of chemical pesticides, entomologists have resorted to alternative means to control insect pests, such as physical and biological methods. One of these methods is the use of insect growth regulators (IGR), which are compounds manufactured by humans similar to those in insects and overlap when used with regulators. Insect growths inside the insect hinder its work and lead to its death. (Hamoud, 2012). Hussein and Nihad (2014) studied the effect of using cinnamon, ginger, nutmeg, and coriander, as these plants had a clear effect on the insect's productivity. The results of Sanaa's study (2014) showed that all plant powders used in the experiment have a repellent property. One of the selected microbes that has been widely used for the production of bioinsecticides is a strain of member species of *B. bassiana*. This research is expected to contribute preliminary data about the ability of entomopathogenic fungal infection. Especially about the age of insect death after infection states that entomopathogenic fungi that match the host will produce a good combination of enzymes to be able to penetrate that depending on several pathogenicity factors, including host suitability and physiological properties of the fungus [Besheli et al, 2000]. This study aims to determine the effectiveness of *B. bassiana* fungus against warehouse pests from the order Coleoptera, *Tribolium castaneum.*

MATERIALS AND METHODS

Mass Rearing of Red Flour Beetle:

Rice infested with the red flour beetle was obtained from local markets, from which 10 pairs (10 males + 10 females) were isolated and added to an amount of 500 grams of healthy rice in order to obtain a pure colony of the insect. They were then placed in clean plastic bottles and covered with a plastic cap. Tie cloth or tie it with a rubber band and place it at laboratory temperature.

Preparation of Concentrations of Fungus *Beauveria bassiana:*

An appropriate number of concentrations are prepared by taking a weight of the *Beauveria bassiana* fungus through a pipette, adding the appropriate volume of distilled water, then mixing it with a glass rod until the concentrations become (1,2,3,4%).

Treating the Larvae of the Red Flour Beetle with the *Beauveria bassiana* fungus:

To treat the larvae of the red flour beetle, 15 Petri dishes were used, where intact rice was placed in all the dishes, and then the first instar larvae of the red flour beetle were placed, which were isolated from the original colony using forceps. The number of larvae in each dish was 10, and after filling the dishes with intact rice and the instar larvae. The first is to take 3 dishes and spray with a concentration of (1%), leaving one dish for control, 3 of them with a concentration of (2%), leaving one dish for control, and 3 of them with a concentration of (3%), leaving one dish for control. Likewise, 3 of them were sprayed with a concentration of (4%), leaving one dish. For control, after the dishes dry, they are closed and numbered, the date, concentration, and number of larvae are written, and the life rate of the larvae is revealed over the course of an entire week. The same is true for the advanced larvae when treated with this pesticide. We use the same method but simply put the advanced larvae in the dishes instead of the first instar larvae.

Statistical Analysis:

The analysis was done according to the model of practical experiments and with a complete design: factorial experiment with a completely randomized design, then the least significant difference (L.C.D) under the level of 0.05 was used to indicate the significance of the results (Al-Raawy and Wakhalf Allah, 2000).

RESULTS AND DISCUSSION

The results of Table (1) indicated that the mortality percentages of first-instar larvae increased proportionally with the increase in the concentration used. The highest mortality percentages of larvae were recorded when treated with a concentration of (4%). The mortality percentages increased as the insects continued to be exposed to the aqueous extract of the *Beauveria bassiana* fungus, and the highest mortality percentages were recorded on the seventh day. The highest value of larval mortality was 90% on the seventh day when treated with a concentration of (4%). The statistical analysis showed that there was a significant difference in the larval mortality percentages of the control group and the mortality percentages of larvae treated with the aqueous extract of the fungus and at all concentrations used in the experiment. The cause of the mortality of the first instar larvae of the flour red beetle may be attributed to the toxic effect of the active substances present in the fungus extract, which leads to decomposition. The lining of the middle alimentary canal causes holes in it and the larvae are unable to digest and absorb food, resulting in the death of the larvae. Many studies, including Singh and Prakash (2002), have proven that early larval stages are more sensitive than late larval stages to many plankton and fungal filtrates. The results of Rizwan (2019) indicated that the highest concentrations of *B. bassiana* are more effective for the virulence and progeny suppression of T. castaneum. Idrees (2021) showed that the first instar larvae of the corn stem borer Sesamea cretica and the sockeye insect Batrachedra amydaraula were more sensitive to infection with the Beauveria bassiana fungus than the other instars

Table 1: Effects of different concentrations of the fungus *B-bassiana* filtrate on The mortality percentages of first instar larvae of the red flour beetle *T. castanium* .

Conc. (%)	Mortality % after one day	Mortality % After two days	Mortality % after three days	Mortality % after four days	Mortality % after five days	Mortality % after six days	Mortality % after seven days
0 .0	0 .0	0 .0	0 .0	0 .0	0.0	0 .0	0.0
1	30	40	50	50	50	60	60
2	30	30	40	50	50	60	70
3	30	30	30	40	40	50	70
4	30	40	50	50	60	70	90
LSD 0.05	11	14	22	21	22	28	24

The results of Table (2) showed that high mortality percentages occurred in the fourth instar larvae of the red flour beetle, where the highest mortality percentages were recorded when treated with a concentration of (4 %) of the aqueous extract of the fungus. The mortality percentages increased at each concentration used in the experiment over time, as the highest mortality percentages were recorded on the seventh day of the experiment, and the highest mortality percentage was 80% when treated with a concentration of (4 %) on the seventh day of the experiment.

The statistical analysis showed that there was a significant difference in the larval mortality percentages in the control group and the mortality percentages of larvae treated with the aqueous extract of the fungus at all concentrations used in the experiment.

Table 2: Mortality rates of fourth instar larvae of the red rust flour beetle *G. mellonelle* treated with different concentrations of the fungus *B- bassiana*

Conc. (%)	Mortality % after one day	Mortality % after two days	Mortality % after three days	Mortality % after four days	Mortality % after five days	Mortality % after six days	Mortality % after seven days
0.0	0.0	0.0	0.0	0 .0	0 .0	0.0	0 .0
1	10	10	20	20	30	30	40
2	30	30	40	40	50	50	60
3	40	40	50	50	60	60	70
4	40	50	50	60	70	80	80
LSD 0.05	25	27	27	25	25	18	16

Vyas (2006) indicated that the *L.giganteum* fungal filtrate had a greater effect on the three larval instars of *An.stephensi mosquitoes*, while it had less effect on the fourth instar larvae. Suman (2008) indicated that the first instar larvae of *An.stephensi* and *Cx.quinquefuasciatus* mosquitoes were more sensitive to Treatment with *M. anisopliae* fungal filtrate compared to the fourth instar, which was more resistant to the fungal filtrate. Al-Jubouri (2008) also noted that mortality percentages increase with increasing concentration of fungal filtrate. Scholte (2003) explained the reason for the increase in larval mortality percentages by increasing the concentration of the fungal filtrate and pointed out that the larval immune system can defend the body only at low concentrations, and when the concentration increases, the immune system loses its efficiency. Lillehoj (1982) mentioned that fungi vary in their secretion of enzymes and mycotoxins, which may penetrate through the insect's body wall, where these substances destroy or disrupt some tissues or may affect the growth and development of the insect. The deaths in the current study can be explained by the fact that the secondary metabolites of the fungus have the ability to interfere with the immune system and cause changes in the host’s behavior, such as reduced activity, paralysis of the insect, reduced nutrition, and changes in tissue structures, thus causing rapid death of the host (Charnley, 2003).

Declarations:

Ethical Approval: Ethical Approval is not applicable.

Competing interests: The authors declare no conflict of interest.

Contributions:I hereby verify that all authors mentioned on the title page have made substantial contributions to the conception and design of the study, have thoroughly reviewed the manuscript, confirm the accuracy and authenticity of the data and its interpretation, and consent to its submission.

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Availability of Data and Materials: All datasets analysed and described during the present study are available from the corresponding author upon reasonable request.

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