



STUDYING THE POSSIBILITY OF USING FUNGUS *BEAUVERIA BASSIANA* IN THE BIOLOGICAL CONTROL OF RED FLOUR BEETLE *TRIBOLIUM CASTANEUM*

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Abstract

Fungus *Beauveria bassiana* was used to determine efficiency of the killing against the Red flour beetle *Tribolium castaneum* in its different larval stages, pupa and adult stages. Three concentrations of the fungus extract were used to determine the most effect concentration of the fungus extract on mortality percent of the insect. The results showed that the concentration of the fungus extract had a significant effect on Insect in all stages of life and this percentage increases with the increasing of mushroom concentration (*B. bassiana*) extract.

Keywords: Fungus extract, *Beauveria bassiana*, biological control, Insects, *Tribolium castaneum*

Introduction

Protein-rich wheat grains are the most important grains and are the main food for humans, wheat is preferable to other grains in the manufacture of bread, which is the main food of a very wide part of the peoples in the world, especially the Arab peoples. Wheat flour beetles *Tribolium castaneum* feed on wheat grains and flours reducing the quality of these grains, change their taste and smell significantly and change many of their qualities and properties (Mohammed and Waddah, 2014). The stored grain and its products are also exposed to many pests that cause significant economic damage, about (10% -11%) of the world's stored grain crops (Al-hadidi *et al.*, 2010), These insects are spread all over the world as whole insects and larvae feed on grains and their products, especially flour, grit and bran, causing unpleasant smell, as well as changes in weight and contaminants associated with infection. There are many ways that man invented to avoid the infection of these insects in order to reduce the losses resulting from this infection, such as plant extracts and some chemical pesticides. Also used dieters prevention, sexual stimulants and attractants to prevent the infection of these beetles (Sulaiman, 2005). In this study, biological control was used as a basis for the eradication of this pest by the use of fungal pathogen *Beauveria bassiana*, that has a wide range in the infection of many insects in addition to the safety towards the human being non-pathogenic and doesn't affect human health, has no effects on the environment and doesn't cause harmful pollution to humans or their property (Lord, 2009; Fagad *et al.*, 2005). Therefore, the trends towards the use of such organisms that are not harmful to human and animal health and at the same time have a good impact against grain insects and stored materials. Pesticides often create large problems due to their widespread use against many species of insects, also resistance to insecticides due to varying periods of chemical pesticides, pollution that can result from this use, and the persistence of long-term pesticides in the environment due to extensive uses in Control. The beetle insect spread widely in Iraq and caused extensive contamination in the grain stores throughout the country. It caused the loss of large amounts of rice, wheat, corn and other economic grains with the participation of a number of

other insects associated with it such as beetles red flour and rice pest and other insect (Clemente *et al.*, 2003). Duval *Tribolium confusum* is one of the most important insect pests in Iraq and many parts of the world, attacking many food products such as flour, cereals, meals, biscuits, chocolate, spices, processed materials, dried food, nuts, and museum models, (Weston and Rattingourd, 2000). The flour infected by this insect has a distinctive smell and moldy taste as a result of insect secretions of (quanin) and flour loses many of its properties such as viscosity and rubber making it unusable for human use (Karunakaran *et al.*, 2004). (Fogliazza and Pagani, 2003) mentioned that the (Duval flour beetle) is one of the most important insects affecting dough made from wheat flour and has a negative impact on the quality of bread. The aim of this study is to determine the level of killing efficiency for the red flour beetle in different stages of its life using insect infected with fungus *Beauveria bassiana*.

Materials and Methods

The insects were collected from the flour found in the flour shops in Ramadi city (west of Iraq) and after checking the different stages of the insect, placed in a plastic box (10 x 30 x 5 cm 3) containing (1/2) k. of flour and (1/2) k. of wheat grain. 25 ± 2 and (50-70%) humidity in order to be the main source for the insects using in this experiment. Petri dishes were used to breed insects and treat insects with the fungus extract. (5) gm. of wheat were placed in each dish, (10) insects were placed and (4) replicates were used for each of the concentrations used in the spores used in the study (24x710, 24x610 and 24x510) allow the comparison group (control) without adding the fungus extract and only spraying with distilled water. To study the killer effect of the fungus spores and the best concentration of the treatment, insects were placed for each concentration in a food-free Petri dish and sprayed with (3) ml of the fungus extract and specific concentration and left for (5) minutes then returned to media taken from it , left in the incubator at (25 ± 2) °C, the readings of the kill rate at each concentration after 24 hours, 48 hours and 72 hours, and 4, 5 days later, the percentage of killing in different concentrations and control group were calculated and the results were corrected according to (Abott,1925) equation:

$$\text{percentage of mortality} = \frac{\text{treated present} \times \text{control present}}{100 - \text{control present}}$$

Isolation of fungi used in the control *Beauveria bassiana* obtained from the Faculty of Agriculture-University of Baghdad and isolated, isolation of the Agriculture- agar media containing potato PDA and prepared according to the ratios used in the growth of fungi and the work of the main extract of the fungus by adding (10) ml of distilled water to Petri dish containing colony of growth mushroom for (1) week and calculated the number of spores using the (Hemosetometer) and using the following equation:

$$\text{Number of spores} = \frac{N}{80} \times 10^6 \times 10$$

Statistical analysis

The experiment was designed on the basis of random design, and the results were analyzed using the SPSS statistical program and using the LSD test to compare the means with a significant level of 5% (Ali and Bala Bhaska, 2016; Al-Rawi and Khalafallah, 2000).

Results and Dissection

The results of this study showed that the *B. bassiana* had a clear effect on the stages of the red flour beetle and this effect increased with the increasing of concentration used in the treatment until the killing rate reached 100% at second and third concentrations of the fungus spores extract in less than 5 days. Table (1) indicate that the larval stages were influenced by the fungal extract more than the pupa ,the second and third phases (first larval stages) were more affected than the fourth and fifth stages (last larval stages), this effect is directly proportional to the concentration increasing of the fungal extract, as killing percentage reached 100% in the second and third concentrations in just (4) days and this indicates to the fungus effectiveness and the killing speed of the insect as a result of penetration of the fungus into the insect body in addition to the toxic secretions resulting from the fungus, which caused the destruction of insects in a short period of time, this is what appeared in the third concentration where the killing percentage 100% through only (4) days for all larval stages and pupa.

for the pupa stage, it was found that the fungus was very effective in eliminating the insect in this phase,

especially in the second and third concentrations, while the effect was less in the extract of the fungus at the first concentration, but the killing speed was less than larvae and may be due to the slow appearance of symptoms on the pupa, which can be distinguished from change of color when dying. The results of the statistical analysis showed significant differences in the killing rates of the three concentrations used at (p 0.05), Table (2) showed insect mortality in adult stages as a result of exposure to the three concentrations and this has been shown that concentration of the fungus had a significant effect on the insect's killing. The increase in the killing rate was proportional to the increasing of the concentration of fungus extract. This may be due to an increase number of active units of fungus presented with the extract.

Results also showed a significant difference at (p 0.05) between the third concentration and each of the first and second concentrations, while the differences were not significant between the first and second concentrations and the highest rate of killing on the (fifth day) of all concentrations, and it was found that the fungus *B.bassiana* had a good efficiency in the killing of the red flour beetle at the larval and pupa stages as well as the adult insect, this is in line with the findings of (Abdullah, 2013) who found that the extract has a great effect on the red flour beetles at all stages. Table (3) shows that the third concentration was the highest in the mortality percentage of larval and pupa stages. This is consistent with many studies that indicated to the most important factor in intensity of infection (temperature and extract concentration using in control (Sabour, 2002 and Scholte *et al.*, 2004).

Table (4) showed significant differences in mortality rates between different larval stages and pupa as well as the effect of the three concentrations used in the study. This indicates that the time should be determined to apply the control in the insect stages as well as the accuracy of selecting the appropriate concentration to success the control, and not giving the opportunity of primer stages from reaching to adult phase Which is the most resistant than other phases, this is due to the hard body and The presence of sheath wings, which gives it more resistance to resistance of the fungal extract.

Table 1 : Percentage of larval and larval stages mortality rate of red beetle exposed to three different concentrations of *Beauveria bassiana*

Extract conce.	Phases	Mortality percentage				
		After 1 day	After 2day	3day	4day	5day
24x 10 ⁵	First larva	20.8	50.2	80.5	90.8	90.8
	Last larva	10.6	40.0	80.3	80.3	80.3
	Pupa	0	0	0	0	40.0
24 x10 ⁶	First larva	0	50.6	60.5	84.4	100
	Last larva	20.3	55.5	68.2	100	
	Pupa	0	10	60.0	80.3	100
24 x10 ⁷	First larva	30.5	50.5	60.6	100	
	Last larva	30.5	50.5	8.80	100	
	Pupa	0	40	80	100	
control	First larva	0	0	10	20.0	20.0
	Last larva	0	0	0	15.6	15.6
	Pupa	0	0	0	0	0

Table 2 : Percentages of the full-scale destruction rate of red beetle exposed to three different concentrations of *Beauveria bassiana*

Extract conce.	Phase	Mortality percentage				
		1 day	2 day	3 day	4 day	5 day
24x 10 ⁵	adult	0	0	33.3	40.6	50.4
24 x 10 ⁶	adult	0	20.5	40.6	58.4	58.4
24 x10 ⁷	adult	10	25	60.2	80.4	90.5
Control	adult	0	0	0	0	0

Table 3 : Mean (+ standard deviation) Insect mortality in larval and larval phases exposed to different concentrations of *B.bassiana* extract

Phases	Conce.	Mean	Std. Deviation	N
First larva	First	66.6200	30.54132	5
	second	59.1000	38.34032	5
	Third	68.3200	30.88182	5
	control	10.0000	10.00000	5
	Total	51.0100	36.47540	20
Last larva	First	58.3000	31.86762	5
	second	68.8000	33.45288	5
	Third	72.3600	30.94064	5
	Control	11.0000	10.24695	5
	Total	52.6150	36.17762	20
Pupa	First	8.0000	17.88854	5
	second	50.0600	33.64078	5
	Third	64.0000	33.35897	5
	control	.0000	.00000	5
	Total	30.5150	30.47402	20
Total	First	44.3067	26.96777	15
	Second	59.3200	32.69634	15
	third	68.2267	33.10031	15
	Control	7.0000	4.21954	15
	Total	44.7133	30.47618	60

Table 4 : Analysis of variance for the effect of insect phase and the concentration of fungal extracts on insect loss rates

Source	Sum of Squares	Df	Mean Square	F	Sig.	
Interference	Hypothesis	119956.931	1	119956.931	10.962	0.045
	Error	32830.387	3	10943.462		
Phase	Hypothesis	6073.540	2	3036.770	3.385	0.041
	Error	48440.622	54	897.049		
Conce.	Hypothesis	32830.387	3	10943.462	12.199	0.000
	Error	48440.622	54	897.049 ^b		

Conclusions

In this experiment, the killing efficiency of the red flour beetle *Tribolium castaneum* was determined in different life stages using *Beauveria bassiana* extract with three concentrations, from the results mentioned above, the following can be inferred: concentration of the fungus extract has a significant and clear effect on the percentage of red flour beetle mortality at all stages life. This percentage increases with the concentration of mushroom extract, this is proportional to the concentration of the extract, In other words, when the fungus extracts concentration increases, the rate of mortality will also increase.

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