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The Role Of Beauveria Bassiana In The Biological Control Of The Different Stages Of The Clover Weevil Hypera Postica

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Abstract

A laboratory study was conducted to evaluate the effect of the biological resistance factor Beauveria bassiana on the different roles of the clover weevil Hypera postica using three concentrations (1×10^2, 1×10^4 and 1×10^6) spore/ml. The results of the study showed that the concentrations used differed in the rates of laboratory fatality of larvae, pupae and adults of the clover weevil Hypera postica. The results also showed that the mortality rates increase with increasing concentration, as the concentration 1 x 10^6 spores/ml recorded the highest mortality rate compared to the other studied concentrations, after 96 hours of treatment, as the mortality rates reached (74.8, 72.9, 70.4, 69.7, 50.3 and 42.1) % for the first larval instar, second larval instar, third larval instar, fourth larval instar, pupa and adult respectively Compared to the lowest mortality rate recorded 6.873 % at the concentration of 1 x 10^2spore/ml after 24 hours of treatment.

Keywords: biological effect ,Hyperapostica , Beauveria bassiana

Introduction

The clover weevil Hypera postica, is one of the most important species of beetles of the Family Curculiono idea that can be found in alfalfa fields all over the world where it poses a devastating threat to alfalfa production, The insect is characterized by being diurnal, and the larval and adult stages are the most harmful to the plant (Maund and Hsiao, 2012) , It starts feeding on the alfalfa crop by ripping off the leaves, leaving holes in them, and transforming the leaf shape into a feathery appearance, causing losses in the plant estimated at about 15%, due to its high reproduction rate and the speed of its nutrition and growth, and it has one generation per year (Kingsley et al., 1993).

To reduce the effects of this insect, several means have been used, the most important of which are chemical pesticides, which represent the main method in combating this insect, as chemical pesticides are the most common in controlling insect pests, but it is a major factor in reducing the quantity and quality of human food, and its role in stopping the damage of agricultural pests cannot be canceled. It is

known that pesticides cause damage and risks to the health of humans and their domestic animals, and the disruption they cause in the natural balance due to the presence of vital enemies of pests (Radcliffe and Flanders, 1998) , Therefore, the researchers directed towards the use of biological resistance elements to protect the product from the toxic residues of the chemical compounds used.

In recent years, many researchers have focused on the use of alternatives to control many insect pests that have a detrimental effect on economic plants, including plant powders and biological control agents , With regard to this topic, the use of Beauveria bassiana is one of the most important elements of biological control, due to its multiple mechanisms in controlling insect pests.As well as studies that indicated that this fungus does not produce toxic substances for plants, does not cause negative effects in the environment, and has no parasitic ability on humans and animals (Twij et al., 2008), Therefore, the study aimed to use the fungus Beauveria bassiana in biological control of the economically important clover weevil Hypera postica in alfalfa fields.

Materials and methods

Breeding insects

Collected of Hypera postica larvae and adults from some cultivated fields in Samawah governorate in southern Iraq and transferred to the laboratory. The insect was bred on Medicago sativa crop to obtain the adults of the insect.

Young leaves were collected from the fields referred to in the previous paragraph and placed in plastic containers with a diameter of 5 cm and a height of 7.5 cm, at a rate of three containers for each treatment. And with three replications for each treatment, and the alfalfa leaves most affected as a result of feeding the insect are replaced whenever the need arises.

The insect was reared at a constant temperature of 25 ± 2 ° C using the incubator and at its base glass containers filled with water with a diameter of 19.5 cm and a height of 3.5 cm were dissolved in it 30 g of KoH in 100 ml of water to obtain a constant relative humidity (70 ± 5%). Thermo hygrometer was used to ensure the stability of temperature and relative humidity, and the incubator was provided with a 20 w light source with a timer to give a fixed light duration of 16 hours of light and 8 hours of darkness. The incubator was used under the same conditions in all subsequent laboratory experiments.

The eggs of the insects were collected from the places where they were laid by the adults and they were placed 24 hours after being laid by the female in transparent, white cylindrical plastic cups, dimensions 5 x 7.5 cm, and placed inside the cups a filter paper moistened with water to provide the necessary moisture for the eggs to hatch, The cups were covered with a mesh cloth and tied with a rubber rope to prevent the larvae from emerging after hatching. The larvae were transferred to the special

containers for rearing them as indicated previously. The virgins were isolated when the fertilization was completed in the aforementioned containers separately and with three replications for each treatment. When the adults came out, each pair (male and female) was placed from The newly emerged adult insects were placed in the prepared containers for breeding and in three replicates and prepared for subsequent studies (Lu.F et al., 2013).

Preparation of B.bassiana concentrations

Pre-diagnosed isolates of B.bassiana were prepared from Plant Protection Laboratories / College of Agriculture / University of Al-Muthanna in Iraq. Three concentrations of fungus (1 x 102, 1 x 104 and 1 x 106) were prepared, spores/ml were added to each concentration a few drops of Tween 80 solution at a concentration of 0.10. % as a moisture preservative and diffuser. Spores / ml of the original solution were measured according to the method of Goettel and Inglis (1997), which depends mainly on the following equation: -

Volume for (A) cell = Mean no. × Conversion factor

Pathogeni city test of Beauveria bassiana on larvae, pupae and adults Hypera postica

For the purpose of restricting the movement of insects and in preparation for treating them with fungi, all insect ages were transferred to dishes. All dishes were placed in the refrigerator at a temperature of 4 °C for 1 hour, after which the dishes were taken out and the insects were sprayed in their glass dishes with the mentioned fungal suspension according to their concentrations by means of a small hand sprayer (1.1) liters Equipped with a compressor piston and containing 300 ml of mushroom spores concentration, while three other dishes were sprayed with sterile distilled water only for the purpose of comparison. The sprayed insects were left on blotting paper for one hour, after which they were gently transferred to glass test tubes 20 cm long and 3.5 cm in diameter, containing inside them at the base a medical cotton moistened with sterile distilled water to saturation and covered with a piece of blotting paper, in which five sterile pieces of a plant were planted. Medicago sativa for nutritional purpose. All tubes were transferred to the electric incubator under the same conditions mentioned above. The readings were taken after 24, 48, 72 and 96 hours of treatment. The effectiveness of fungal concentrations was calculated according to Abbot's equation (1925). And as follows:

$$P = \left(\frac{C - T}{C} \right) \times 100$$

Results

Effect of B. bassiana concentrations on the mortality of incomplete roles and adults of H. postica at different time intervals (hour)

First larval stage

The results showed that the different concentrations of B. bassiana at different time periods had a significant effect on the percentage of mortality of the first larval stage of H. postica, It is clear from Table (1) that the highest fatality rate was 74.8% at concentration 1 x 106 spore/ml for the time period of 96 hours and the lowest mortality rate was 37.4% at concentration 1 x 102 when exposed for 24 hours. At different time periods as well as overlap.

Table (1): Effect of different concentrations of B. bassiana on the mortality of the first larval stage of H. postica at different time periods (hour)

fungi concentration ((spore/ml	Percentage of mortality per / hour				Overlap between concentration and time
	24	48	72	96	
$10^2 \times 1$	37.4	43.3	58.8	69.8	52.3
$10^4 \times 1$	41.9	54.6	65.7	72.9	58.7
$10^6 \times 1$	52.1	57.0	67.0	74.8	62.6
General average time effect	43.8	51.6	63.8	72.5	
L.S.D 0.01	2.4				4.1

Second larval stage

The different concentrations of the fungus B. bassiana and within different time periods had a significant effect on the percentages of deaths of the second larval stage of the insect, and its highest rate was 72.9% at concentration 1×106 in the exposure period of 96 hours, and it decreased to its lowest rate of 42.2% at concentration 102×1 at 24 hour exposure (Table 2) , As well as for the interaction between the concentrations of the biological fungus B. bassiana and time, where it was observed that there is a significant difference that increases with the increase in the time period and the concentration of the fungus used, which reached a maximum of 63.6% at the concentration 1 × 106 in the exposure period of 96 hours and this percentage

decreased at the concentration 1 × 102 when exposed for 24 hours. hour, reaching 58.2%.

Table (2): Effect of different concentrations of B. bassiana on the mortality of the second larval stage of H. postica at different time periods (hour)

fungi concentration ((spore/ml	Percentage of mortality per / hour				Overlap between concentration and time
	24	48	72	96	
10^2×1	42.2	58.5	62.7	69.4	58.2
10^4×1	45.9	60.8	65.5	71.5	60.9
10^6×1	48.3	64.1	69.2	72.9	63.6
General average time effect	45.4	61.1	65.8	71.2	
L.S.D 0.01	0.497				1.172

Third larval stage

The results of the study showed that the use of different concentrations of the fungus B. bassiana led to a significant and clear increase in the percentage of mortality of the third larval stage in different time periods, the highest rate reached 69.1% at concentration 1×106 and during a period of time 96 hours and the lowest was 39.6% at concentration 1×102 and the duration is 24 hours, the results of the statistical analysis showed that there were highly significant differences between the rates of the percentage of mortality of the third larval stage of the insect at the same concentration of the fungus when the insect was exposed to different periods of time as well as within the different concentrations at the same time period (Table 3).

Table (3): Effect of different concentrations of B. bassiana on the mortality of the third larval stage of H. postica at different time periods (hour)

fungi concentration ((spore/ml	Percentage of mortality per / hour				Overlap between concentration and time
	24	48	72	96	
10^2×1	39.6	48.1	60.6	67.2	53.8
10^4×1	45.1	57.4	63.3	69.8	58.9
10^6×1	47.3	62.3	66.1	70.4	61.5
General average time effect	44.0	56.1	63.3	69.1	
	0.430				0.859

Fourth larval stage

The results at different concentrations of B. bassiana and different time periods indicate that there are significant differences in the percentages of mortality of the

fourth larval stage of the insect, It is noted from Table (4) that the highest percentage of insect mortality reached 69.7% at concentration 1 x 10^6 in a period of 96 hours and decreased to 37.2% at concentration 1 x 10^2 in a period of 24 hours, There are also highly significant differences in the percentages of insect mortality at the interaction between the concentration of the fungus and the time period, reaching a maximum of 59.8% at the concentration 1 x 10^6 in a period of 96 hours (Table 4).

Table (4): Effect of different concentrations of B. bassiana on the mortality of the fourth larval stage of H. postica at different time periods (hour)

fungi concentration ((spore/ml	Percentage of mortality per / hour				Overlap between concentration and time
	24	48	72	96	
$10^2 \times 1$	37.2	46.5	59.6	63.3	51.6
$10^4 \times 1$	43.1	53.1	62.2	65.4	55.9
$10^6 \times 1$	45.6	59.4	64.8	69.7	59.8
General average time effect	41.9	53.0	62.2	66.1	
	0.554				1.108

pupa stage

It is noticed from the results of Table (5) that there are few significant differences between the concentrations 1×10^4 and 1×10^6 at 24 hours, where they reached 23.1% and 23.8%, respectively, Whereas, significant differences were found for the other treatments, reaching a maximum of 50.3% at concentration 1 x 10^6 in a period of 96 hours, followed by treatment with a concentration of 1 x 10^4 and in the same time period, which amounted to 48.7%.

Table (5): Effect of different concentrations of B. bassiana on the mortality of the pupa stage of H. postica at different time periods (hour)

fungi concentration ((spore/ml	Percentage of mortality per / hour				Overlap between concentration and time
	24	48	72	96	
$10^2 \times 1$	20.4	28.5	41.2	46.9	34.2
$10^4 \times 1$	23.1	34.9	42.6	48.7	37.3
$10^6 \times 1$	23.8	38.6	44.9	50.3	39.4
General average time effect	22.4	34.0	42.8	48.6	
	0.548				1.052

Adult stage

The results of Table (6) show that the different concentrations of B. bassiana at different time periods had little effect on the percentage of adult mortality compared to the four larval stages of the insect, It was found that the highest percentage of adult mortality of the tested insect was 42.1% for concentration 1 x 10^6 at 96 hours and decreased to 12.5% for concentration 1 x 10^2 for 24 hours (Table 6). The results of the same table also indicated that there are significant differences in the percentage of mortality, Adults within the same concentration and for all time periods tested.

Table (6): Effect of different concentrations of B. bassiana on the mortality of the adult stage of H. postica at different time periods (hour)

fungi concentration ((spore/ml	Percentage of mortality per / hour				Overlap between concentration and time
	24	48	72	96	
$10^2\times1$	12.5	20.6	31.7	38.6	25.8
$10^4\times1$	16.2	26.2	32.4	41.5	29.0
$10^6\times1$	17.3	28.5	34.4	42.1	30.5
General average time effect	15.3	25.1	32.8	40.7	
	0.695				1.390

Discussion

The results obtained from the experiment indicated that the 1×10^6 spore/ml concentration of B. bassiana was superior to the mortality of the four larval roles of H. postica compared to the other concentrations of the used fungus, From this, it is clear that focus and lighting have an important role in increasing the percentage of insect mortality, and there is a direct relationship between concentration and lighting and rates of mortality rates where the higher the concentration of the fungus and the lighting, the higher the percentage of insect mortality (El. Zoghby, 2003),The reason may be due to the growth of fungal hyphae and penetration of the cuticle layer and spread inside the body of the larvae and the secretion of the enzyme lipase, which works on the digestion of fat tissue, as well as the effect on the plasma membrane of fat cells, which leads to their decomposition and fusion with each other, forming a large gap of varying size within the tissue. cells together This is consistent with the findings of Miranpuri and Khachattourians (1991) when studying the changes associated with the roles of Aedesaegypti infected with B. bassiana , Where he noticed the effect of the fungus on the muscle layers of the alimentary canal, which led to a change in the thickness of the muscle layers compared to the control treatment, as well as the loss of the muscle layer of its features, and this indicates the depletion of the fungus from the alimentary canal ,These results are in agreement with what was mentioned by Hala and Hossam El-Din (2012) in their study of the effect of B. bassiana on the histological changes of larvae and adults of Culexpipienspipiens.

This is due to the fact that the fungus has penetrated the cuticle layer, the epidermis and the fatty bodies, causing major tissue changes in them, then invading the entire digestive system and turning the insect into a white mass after 120 hours of infection and thus its death. This is consistent with the findings of (Fagad and Lomer, 2005).

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