حساسية حشرة *Ephestia cautella* (Walk.) للفطر الممرض (Zimm.) *Verticillium lecanii*

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الملخص

هدفت الدراسة الى تقييم فاعلية تراكيز مختلفة من العالق البوغي للفطر Verticillium lecanii في الادوار الحياتية (بيض ، يرقات ،عذارى وبالغات) لحشرة العث Ephestia cautella ، تحت ظروف المختبر (بدرجة حرارة 25 ± 1 مئوية ورطوبة نسبية 65± ٥ % وفترة ضوئية ١٤ أضاءة : ١٠ ظلام) . أظهرت النتائج تأثر كافة الادوار الحياتية لحشرة العث عند تعرضها للرش المباشر بتراكيز العالق البوغي المختبرة (١٠٧,105,103) بوغ / مل بعد (٢ ، 5و ١٠) يوم من معاملتها مقارنةً بمعاملة السيطرة.

بينت نتائج هذه الدراسة تفوقاً معنوياً في نسب هلاك بيض الحشرة بعد ٢ يوم من المعاملة عند كافة تراكيز المعلق البوغي المختبرة للفطر V. lecanii حيث بلغت 100% مقارنة بمعاملة السيطرة التي بلغت نسبة الهلاك فيها 0.00% . واظهرت معاملة الطورين اليرقي والعذري بعد ٥ يوم من المعاملة عند التركيز ١٠٧ بوغ / مل أعلى نسبة هلاك اذ بلغت 100% . وحقق التركيزان (105,103) بوغ / مل نفس نسبة الهلاك بعد ١٠ ايام من المعاملة مقارنة بمعاملة السيطرة.

وفي جانب اخر تشير نتائج تعريض البالغات (ذكور و أناث) الى موت جميع البالغات عند المعاملة بالتراكيز المختبرة (١٠٣، 105، 107) بوغ / مل بعد ١٠ يوم مقارنة بمعاملة السيطرة. وبلغت قيمة التركيز القاتل للنصف LC50 للذكور 6.2 x104 بوغ / مل وللاناث 6.5x105 بوغ / مل. نتائج هذه الدراسة أكدت نجاح أستخدام تراكيز (103 ، 105 ، ١٠٧) بوغ / مل من العالق البوغي للفطر V. lecanii كمسبب ممرض في السيطرة على حشرة العث E. cautella (Walk.)في المختبر .

الكلمات المفتاحية: الفطر Verticillium lecanii ، حشرة Ephestia cautella ، نسبة الهلاك ، العالق البوغ

Susceptibility of *Ephestia cautella* (Walk.) to entomopathogenic fungus *Verticillium lecanii* (Zimm.) Preparation

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Abstract

The study conducted in the laboratory of Biology department – College of Science – University of wasit, aimed to evaluate the efficacy of spores suspension concentrations of *Verticillium lecanii* (Zimm.) against (eggs , larvae , pupae and adults) of moth *Ephestia cautella* (Walk.) by direct application to insect at (25 ± 1 °C and 65 ± 5% R.h. and photoperiod 14 L:10 D h(.The results showed that all fungal suspension concentrations (10^3,10^5,10^7) spore/ml affected on different stages of insect after (2 ,5 , 10) day of the treatment compared with control treatment.

The laboratory results revealed that the all concentrations of spores suspension of *V. lecanii* affect the mortality of eggs of moth after 2 day of the treatment and the percentage of mortality were 100%, and shown that the percentage mortality of larval stages and pupae were 100% when they were treated for 5 day at10^7spore/ml followed by the another treatments in concentrations of 10^5,10^3 spore/ml after 10 day of the treatment were 100%.

On the other hand, bioassays conducted to evaluate the effect of direct exposure of adults to the concentrations(10^3,10^5,10^7) spore/ml and it revealed that all adults (males and females) were killed after 10 day of the treatment and the highest percentage of mortality in males and females was100% and the LC50 values of males were 6.2 x10^4 and females 6.5x10^5 spore / ml.

The results of this study were revealed the success and effectiveness of using the *Verticillium lecanii* fungus suspension at (10^3,10^5,10^7) spore/ml as a pathogen in the control of moth *Ephestia cautella* laboratory.

Introduction

The continuous use of insecticides has resulted in several measurable harms to the human and environment, in the integrated management program, researchers have approached several alternate methods and strategies for the safety of pesticides in particular biological control methods using natural enemies (parasitoid, predators and pathogens) in natural biocontrol or applied biocontrol as a key and highly efficient component of numerical density control over agricultural pests worldwide (Khlaywi *et al.,* ٢٠١٤ ; Nico *et al.*,2004 ; Sarwar , 2013 ; Kaewchai *et al.,* ٢٠٠٩ ; Sun *et al.,* ٢٠٠٦). The moth *E. cautella* is one of the major important cosmopolitan pest infesting many hosts, can attack a variety of stored products. The larvae will feed on grains, grain products and dried fruits confectionery, this pest infecting dates in both field and storage houses, In the field, this moth breeds on the upper surface of either the windfall fruits or the harvested date fruits causing damage to the dates from harvesting until marketing in the market and create multiple obstacles to the marketing of Iraqi because close association of date fruits and the presence of this insect (Tariq and Jasser, 2015 ; AL-Taei, 2001). Cosmopolitan *E.cautella* (Lepidoptera : Pyralidae) was first recorded in the U.S.A in 1884 on a field pistachio crop (Cotton, 1950).The larvae of Some Species of *Phycitinae* damaged the stored products imported by British from some countries of the Mediterranean Basin (Aitken,1963). The insect also played a chief role in the infection of leguminous crops in India (Gonzalez, 1989). The larvae were highly efficient and effective in the ravage of dates covered from abroad with selenophane paper and access to dates, harm causing significant economic losses (Cline ,1978). Larvae of this insect cause direct damage to store product by making the product unattractive to consumers and as well contaminate food and produce silken thread that is webbing the products together (Wood and Lass, 1989). The most important organisms used in the IPM programs were fungal insects affecting the population density, most of them belonging to the missing fungi deuteromycota, the most common and successful fungus *M. anisopliae, B. bassiana and V.lecanii* (Scholte *et al.*, 2004 ; lacey *et al.*, 2001; Zimmermann , 2007).

Vereticillium lecanii was used in the control of several insect pests and was used in the control of the *Eurygaster intericeps* (pests of wheat) (Abu Obeid and Ahmed, 2000). *V. lecanii* was one of the most common fungus, as a commercial vaccine of fungus spores produced control Whitefly *Taleeuroides vaporariorum* affecting vegetables (Wang *et al.*,2005 ; Masuda and Kikuchi,1992).The fungal specialization of most

entomopathogenic fungi has been enhanced by the possibility of being used in the field of biocontrol as attacking the target insect, attacking natural enemies as well as its ability to form effective poisons against the target pest, limiting the appearance of resistant strains and leaving no toxic residues (Ekesi *et al.* 2007*;* Montasser *et al.* 2012*;* Hamdy, 2015 ;Castrillo *et al.,* 2005).

Materials and methods

Insect and Fungi Source

The adults of *E. cautella* (Walker) and *V. leucanii* fungus were obtained from the stock colony maintenance in were In tegrated Pest Control Center, Directorate of Agricultural Reseach, Agriculture - Ministry of Science and Technology- Baghdad laboratories. The insect colony was prepared in an incubator kept under constant conditions of temperature, photoperiod and humidity of 25 ±1 ° C , 14 L:10 D and 65±٥ % respectively.

***E. cautella* (Walker) Culture**

Newly hatched larvae of *E. cautella* were confined with the help of a camel's hair brush in cages made of transparent and cubic organic glass, 40 cm in length, open from the top, intended for rearing, with artificial diet of 81% crushed wheat , 12 % glycerine, 6% date molasses, 1% dry yeast). These cages were prepared in Petri dishes (9×1.5) cm. They contain a piece of cotton saturated with water, with an addition of 250 mg tetracycline, then at the ground of the culture cages. A side opening surrounded by a removable plastic cover to sustain the colony. After the growth follow with removable plastic cover to sustain the colony. *E.cautella* adult (male and female) were placed in an open-ended glass cylinder. The top hole was covered a raw cloth with a hole in it and supplied with food (cotton saturated with sugar 10%). In the lower hole, a muslin cloth tightened with a rubber band to ensure good aeration and able cloth that allows the vents to penetrate the eggs is placed into petri dish placed to collect eggs from the female (Ahmed *et al.* 1986).

Preparation of spores suspension

The fungus *V. lecanii* were grown in petri dish 9 cm containing PDA medium at 25 ± 2° C in incubator for two week to obtain fungal colonies and preparation three concentrations of spores suspension of *V.lecanii* (10^3, 10^5, 10^7) spore1 / ml by the aid of haemocytometer.

Laboratory experiments

Effect of spores suspension of *V.lecanii* in eggs hatching

Newly laid eggs of about 1-12 hours age with 20 eggs per replicate with 3 replicates per treatment on filter paper in 9 cm Petri dishes. The eggs were treated with 5 ml of concentrations of spores suspension of *V.lecanii* (10^3, 10^5, 10^7) spore /ml by direct spraying using a 10 mL hand spray. The comparison group was treated with egg spray with only 5 ml of distiller water. All the treatments were placed in an incubator at 25 ± 1 ℃ and followed up and the egg mortality rates were calculated after (2 , 5 , 10) day of treatment with the fungal suspension of each treatment.

Effect of spore suspension of tested fungus in larvae and pupae

10 larvae and 10 newly pupa were treated with 5 ml of concentrations (10^3, 10^5, 10^7) spore/ ml of the *V.lecanii* fungus in a sterile plastic flask 11 cm with a height of 12 cm containing 250 g of artificial diet to feed the larvae and covered the dishes with muslin cloth. The comparison group treated the larvae and pupa with 5 ml of sterile water and the larva and pupa mortality were calculated after (2 , 5 , 10) day of treatment with the fungal suspension of each treatment.

Effect of spore suspension of tested fungus in adults (males and females)

E.cautella (Walker) was treated at 72 hours of newly emerged by 20 adult (10 males x 10 females) per replicate and 3 replicates per treatment in a sterile flask (11 ×12) cm with 250 g of artificial dite was covered the flask with a plastic cover in the middle a hole of 2 cm diameter for aeration is covered with muslin cloth to preventing adult exit and treatment with 5 ml per replicate of the spore suspension of the *Verticillium lecanii* (10^3, 10^5, 10^7) spore/ml Sprayed directly with a 10 mL hand spray according to the treatment of each replicator. The comparison group was treated with 5 ml of D.W. and followed up and counting the number of dead females after (2, 5, 10) days of treatment with the fungal suspension of each treatment. All experiments were "[designed according to the complete randomized CRD

design. The results were analysis according to the SPSS version20, which included the DMRT test to compare the rates in all treatmentsand to determine the significant at a probability level of 0.05. The Probit analysis was used to extract the lethal concentrations of half LC50 (Payne *et al.*, 2003)]".

Results and discussion

Effect of spore suspension of tested in the mortality of *E. cautella* eggs hatching

The results of the study on the effect of the diverse concentrations (10^3, 10^5,10^7) spore / ml of *V. lecanii* in the mortality of *E. cautella* eggs showed significant of all tested concentrations after 2 days of treatment. When comparing with a control treatment (without treatment) **Table 1**. The efficacy of the fungal tested was according to the concentration of the suspension and the period of exposure. *Verticillium lecanii* was recorded dead to all *E. cautella* eggs at all tested concentrations reach 100% after 2 days of treatment. This may be due to the parasitic ability and enzymatic activity of *V. lecanii*. Mehdi (2002) refered to the causes of the effect of entomopathogenic fungus in egg hatching rates due to the ability of parasitic fungus on eggs and its production of chitinase enzyme, which mechanism on the analysis the chitin of egg walls, thus enabling the penetration of the egg and the entry of fungal hyphal growth within the egg and the destruction of their contents with the embryo has died from mycosis. According to the results achieve with the results of a study by Karadaghi *et al*., (٢٠١٤) of the sensitivity of *Culex quinquefasciatus* eggs when treated with concentrations (10^6, 10^7 and 10^8) spore / ml of the fungus *Metarhizium anisopliae*. The treatment was with a concentration of 10^8 spore /ml of fungal suspension the ratio of hatch by 71.30% showing significant difference with other treatments, the ratio of hatching eggs was not significantly differences at (10^6 and 10^7) spore / ml which was 80% and 79.37% , respectively, compared to control treatment 35.03 %. Similar results were obtained by Saleh *et al*., (2010) the efficacy of *V. lecanii* in the early stages of *Agrotis oipsillia*, the highest insect mortality was recorded using *V. lecanii* fungus by 98.0% in laboratory and field 93.0% .A recent laboratory study showed that exposuer of *C. carnea* eggs at the age of one day to the concentration 10^9 spore / ml of the fungal suspension *of M.anisopliae* (Met.) and *B.bassiana*. (Bals.) there was no significant difference in the percentage of eggs after treatment hatching was 27.6% and 26.7%, respectively, and there was an converse

relationship between hatching and concentration value. The higher the suspension concentration, the lower the hatch percentage (Soltan, 2016).

Table 1: Effect of suspension Fungal of *V.lecanii* in the mortality of *E. cautella* eggs

Treatment	Con.	Moratility of eggs % day		
		2	5	10
Verticillium lecanii	١٠٣	**100 b**	-	-
	١٠٥	**100 b**	-	-
	١٠٧	**100 b**	-	-
Control	**0.0**	0.00a	0.00a	0.00a

Effect of spore suspension of tested in the mortality of *E. cautella* larvae

The results of the effect of the suspension for fungal tested on larvae from the first age (newly hatching) revealed that there is a significant difference between the concentrations of tested fungus in their parasitism of larvae and the percentage of mortality in the case 10^7spore / ml was (100%) After 5 days followed by the concentrations (10^3 and 10^5) spore / ml was (82.16 % and 83,41%) respectively also death for all larvae after 10 day of treatment **Table 2** . All concentrations differed significantly compared control treatment was (0.00 %).Death of the larvae caused by the penetration of the hyphae of fungus into the larval cuticle and thus the larval loss of a large amount of the body's water, leading to an imbalance in the conduct of the biochemical reactions within the living tissues and thus death. A similar study to evaluate the effect of some *B.bassiana* (Bals) isolates affected the *Argerosae* insect so that all isolates were effective in larval mortality and that the larval mortality of larvae increased by increasing concentration

(Khosravi 2015). study of laboratory indicated LoVerde *et al*. (2015) is the the effect of three fungal isolates In the control of *Rhynchophorus ferrugineus* to the susceptibility of all tested isolates to larval infection and high mortality percentage.

Table 2 : **Effect of suspension Fungal of *V.lecanii* in the mortality of *E. cautella* larva**

Treatment	Con.	Moratility of larvae % day		
		2	5	10
Verticillium lecanii	10^3	80.87c	82.16b	100b
	10^5	82.30 c	83,41b	100b
	10^7	91.67bc	100c	-
Control	**0.0**	**0.00a**	**0.00a**	**0.00a**

Effect of suspension Fungal of *V.lecanii* in the mortality of *E. cautella* pupae

The results shown significant differences in the mortality of pupae that compared with control group was 0.00%, and there was no significant difference in the mortality of the pupae at the treatment of all the concentrations of the fungus (10^3, 10^5, 10^7) spore / ml was the mortality percentage of lowers after 5 days of treatment reached 100% at 10^7 spore / ml when reached at 10^3 and 10^5 spore / ml was (89.80 %, 90.30%) respectively, but 10 days after treatment the mortality at 10^3 and 10^5 spore /ml was 100%. . When the pupae were exposed at the treatment after 2 days at all the concentrations (10^3, 10^5, 10^7) spore / ml, had a lowers of 68.30%, 69.11 and 87.03, respectively **Table 3**.

Results of this study was agreed with Obeid *et al.* (2014) that *B.bassiana* had a significant effect on the mortality of the pupae low of 50.16% and 40.10% for *Rhizopertha dominica* and *Tribolium castaneum* respectively that compared with control group was 3.33% .

Table 3: Effect of suspension Fungal of *V.lecanii* in the mortality of *E. cautella* pupae

Treatment	Con.	Moratility of pupae % day		
		2	5	10
Verticillium lecanii	١٠٣	68.30 b	89.80cb	100b
	١٠٥	69.11 b	90.30 cb	100b
	١٠٧	87.03 cb	100c	-
Control	**0.0**	**0.00a**	**0.00a**	**0.00a**

Effect of suspension Fungal of *V.lecanii* in the mortality of *E. cautella* Adults (Male and Female)

The results of the treatment of ***E. cautella*** (walk.) For the concentrations (10^3, 10^5, 10^7) spore / ml of the fungal suspension *V.lecanii* showed a correlation between the concentration used and the percentage of mortality and the sex of the treated adult. The mortality in adult increased with increasing Concentrations used. There was a difference in mortality associated with concentration. The mortality of males after 2 days of treatment was(16%, 42% and 94%) for the concentrations of (10^3, 10^5 and 10^7) spore / ml respectively **Table 4**. The statistical differences between the concentrations after 5 days of treatment The mortality were 57% , 74% and 100% at the same concentrations above respectively and The treatment of male showed an clear correlation between the percent of death and the using concentration and the more effective fungus which the LC_{50} value was 6.2×10^4 spore / ml. The concentrations (10^3, 10^5 and 10^7) spore / ml in the female were correlation with concentration after 2 days of treatment was 8% , 51% and 94% , respectively. After 5 days, 25, 75 and 100% were at the same concentrations above LC50 was 6.5 x 105 spore / ml . All concentrations (10^3, 10^5 and 10^7) spore / ml were achieved after 10 days with no significant difference of 100% mortality of *E.cautella* (walk.) adult (male and females) compared no mortality of control treatment.This is Death of the insect may be caused by the the increase in the amount of fungal spores in the concentration of 10^9 spore/ ml and enable the largest number of spores from falling on the body of the insect and the incidence of infection, increasing the spores more effective fungal suspension to cause infection and the ability to secrete the enzymes (protease, lipase and chitinase) analysis of the wall of the insect in addition to fungus proliferation and spread within the body of the insect The length of time of injury depends on the physical structure of the external insect wall . The outer epidermis in the adult (Bekheit and Abo El-Abbas, 2002). The results of the present study are agreement with the founding of Dimbi *et al.* (2013) who found that there is a difference in sensitivity of *Ceratitis* sp.to pathogenic fungi between adults, which showed the sensitivity of females compared with males, including faster death of the female *Ceratitis* sp. Compared to males when treated with suspension fungus *Metarhizium anisopliae*. The results obtained by Salih *et al*. (2010) showed that there were no significant differences between the *B. basiana* (Bals.) concentration in the adult age of the domestic fly (*Musca domestica*). The males (24 hours) 10^5 spore / ml for 11.9 days

and 11.4 days for 10^3 spore / ml and 12.2 days for 10^1 spore / ml compared to 17.9 days for males in the control treatment. For females (24 hours), treated with concentrations of (10^5, 10^3 and 10^1) spore / mL of survival for (11.6, 12.0 and 12.1) days respectively Compared to control treatment, the female of survival was 18.1 days. The results of a study by Abd El- Gawad and Atef (2008) showed the success and effectiveness of using the *Verticillium lecanii* fungus suspension at 10^8 spore / mL and the *C. carnea* (Stephens) larvae second instar together in the control of aphis bean *Aphis craccivora* in Egypt. This study was revealed that the spore suspension of the *Verticillium lecanii* was very effective against *E.cautella* and it can be used as a biocontrol agent against this pest.

Table 4: The lethal concentrations LC50 *and E.cautella* (walk) the mortality of *E. cautella* Adults (Male and Female).

Treatment	Con.	Moratility of % day						LC_{50} spore /ml		Chi square X^2		*P* value	
		After 2day		After 5 day		After 10 day							
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Verticillium lecanii	١٠٣	a ٨	a ١٦	a ٢٦	a ٥٧	١٠٠	١٠٠	٦.٥ × ١٠٥	×٦.٢ ١٠٤	٧. ٨٣	١٦ .٦	٠. ٣٥	٠. ٠٢
	١٠٥	b ٥١	b ٤٢	b ٧٥	b ٧٤	١٠٠	١٠٠						
	١٠٧	c ٩٤	c ٩٤	c ١٠٠	c ١٠٠	—	—						

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