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ENTOMOPATHOGENICITY OF *Beauveria bassiana* (BALS.) VUILLEMIN TO CERTAIN LARVAL INSTARS OF THREE CORN BORERS UNDER LABORATORY CONDITIONS

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ABSTRACT

Laboratory experiments were conducted in 2008 at biological control department, plant protection research institute Sakha Kafr El-Sheikh station to study the efficacy of the fungus *Beauveria bassiana* (Bals.). An isolate collected from *Sesamia cretica* larvae in Kafr El-Sheikh region and cultured on potato dextrose agar medium (PDA) at 25 °C spore suspensions at concentrations of $1x10^7$, $2.5x10^7$, $5x10^7$, $7.5x10^7$ and $1x10^8$ spore/ml were prepared in distilled water containing 0.1% Tween 80. The larvae were individually treated by dipping method in the fungus suspension and bioassayed against the third larval instar of each species, *Ostrinia nubilalis* (Hbn.), *Sesamia cretica* (Led.) and *Chilo agamemnon* (Bles.) incubated at 25±1°C and 75±5 % RH). All tested concentrations induced different mortalities. For *O. nubilalis* L_3, mortality ranged between 5-100 % was reached at 4-10 days with the all tested concentrations LC_{50} value was $1.2127x10^7$ after 12 days while LC_{90} reached $9.7625x10^7$ spore/ml, respectively, meanwhile LT_{50} values reached 12.7, 10.5, 8.6, 8.2 and 7.6 days with the tested concentrations of $1x10^7$, $2.5x10^7$, $5x10^7$, $7.5x10^7$ and $1x10^8$ spore/ml, respectively. As for *S. cretica* L_3, the highest mortality (75-90%) was achieved at 12-14 days post treatment. LC_{50} value was $8.5037x10^7$ while LC_{90} was $15.05x10^7$ spore/ml at the same mentioned period meanwhile, LT_{50} values reached 17.4, 14.8, 11.0, 10.7 and 8.5 days by all tested concentrations.

In case of *C. agamemnon* L_3 the highest mortality reached 80-90 % after 12-14 days of treatment.

LC_{50} value was $1.5884x10^7$, while LC_{90} was $9.8329x10^7$ spore/ml., at 14 days post treatment, LT_{50} values were 15.5, 11.8, 8.8, 8.6 and 8.1 days for the concentrations of $1x10^7$, $2.5x10^7$, $5x10^7$, $7.5x10^7$ and $1x10^8$ spore/ml, respectively.

INTRODUCTION

The entomopathogenic fungus *Beauveria bassiana* (Bals.)Vuill. (Deuteromycotina: Hyphomycetes) is widely regarded as one of the most promising insect biocontrol agents. (Ferron,1981). This fungus was isolated in Egypt from *Hypera brunnerpennis (*Boh.) by El-sufty and Boraei (1987) and from *Torpinota squalida* Scop. by El-Hussini *et al.* (1996) and Sewify (1997) referred to the possibility of using *B. bassiana* against *Sesamia cretica* Led. To produce conidia of *B. bassiana* for laboratory experiments and biological control measures; Müller Kögler and Samsinakova (1969) cultured the fungus on malt extract peptone agar . El-Sufty (1983) cultured the fungus through six successive transfers on two media. Pandit and Som (1988) found that potato dextrose agar medium was most suitable for the maintenance of *B. bassiana* culture. Amalin *et al.* (1991) evaluated the sporlation of *B. bassiana* using three different culture substrates; rice grain, corn stalk and sugar can bagasse. Pham *et al.* (1994) isolated and multiplied the fungus using

Corapec media and Saburo media where optimum growth of the fungus was at 25-30 °C and 70-85 % RH.

The effectiveness of the fungus against several species of insect pests was studied by different authors (El-Sufty *et al.,* 1986; Aguda *et al.* 1987; Boiteau, 1989; Busoli *et al.,* 1989; Bing and Lewis, 1991; Chiuo and Hou, 1993).

Harold *et al.* (1957) gave the first suggestion for the use of *B. bassiana* as a biocontrol agent against the European corn borer ,they observed a high mortality exceeding 90% among a lot of these larvae the same authors supported their suggestion adding that the early three larval instars are internal feeders on the succulent tissues of the leaf, which afford an ideal environment for fungus development.

The pink borer, *Sesamia cretica* Led. (Lepidoptera: Noctuidae) is one of the most important corn borer in Egypt. Insecticides were extensively used to control the pest in maize fields (Ahmed and Kira (1960), Mostafa (1981), Semeada (1998) and Metwally (2000) who found that the maximum infestation with *S. cretica* in Kafr El-Sheikh region was that of maize sown in April plantation. The Europeancorn borer *Ostrinia nubilalis* (Hbn.) is an economic pest in Egypt causing damage to corn especially in July (Nili) plantation. The rice stem borer, *Chilo agamemnon* Bles. (Lepidoptera: Pyralidae) the main pest of rice it causes considerable loss in yield between 3-7% (Sherif,1996).

Topical application and stem injection with African isolates of *B. bassiana* was made by Cherry and Agnassim (2004). Co-application of entomopathogenic fungus with low doses of insecticides is gaining importance in insecticide resistance management in insect pest of crops as a component of IPM program. (Ambethgar 2009). The use of mycoinsecticides has been as inundative agents within chemical insecticides Stefan(2010).

In the present study entomopathogenicity of *B. bassiana* recovered from *S.cretica* on artificial medium (Sewify, 1997) was tested in five different spore concentrations against each of the three species of corn borers on the third larval instar under laboratory conditions.

MATERIALS AND METHODS

Entomopathogenic fungus:

B. basiana used in this laboratory study is an isolate collected from *S. cretica* larvae in Kafr El-Sheikh region according to Sewify (1997). Conidia were cultured on autoclaved potato dextrose agar medium (PDO). Cantwall 1975, Pandit and Som (1988).

Spores were harvested from two weeks old culture grown at $25\pm1^{o}C$. Spores suspended in distilled water were counted using a haemocytometer. Five concentrations of $1x10^{7}$, $2.5x10^{7}$, $5x10^{7}$, $7.5x10^{7}$ and $1x10^{8}$ spore/ml. were prepared.

Larvae of the corn borers:

Egg-clusters of the corn borers were collected from maize fields at Sakha Experimental Station during September 2008 and reared in the laboratory

The newly hatched larvae were reared under laboratory conditions of (25±1 °C and 75±5 % RH) on corn ear silk bundles in glass containers (5x8cm) (Agamy 2002) furnished with filter paper (5 larvae/container) and covered with perforated polyethylene sheet fitted in place by rubber band. The food was renewed each second day until the larvae reached the third larval instar. Each larvae species was represented by 120 larvae divided into six groups, 20 larvae each five groups were treated with different concentrations of *B. bassiana* suspension. The 6th group was treated with distilled water containing 0.1 Tween 80 as a control. The larvae were individually treated by dipping in the fungus suspension for 3 sec. Each five larvae were introduced with fine brush into a Petri dish having corn ear silk bundles. The larvae were examined daily and its food renewed each second day. Mortality was corrected according to Abbott (1925). Values of LC_{50}, LC_{90}, LT_{50} and LT_{90} were calculated using Litchfled and Willcoxon (1949).

RESULTS AND DISCUSSION

Table (1) shows that the 3rd larval instar treated with the five different concentrations of *B. bassiana* resulted in considerable mortalities. The first dead larvae were recorded 4 days after treatment at concentrations of $5x10^7$, $7.5x10^7$ and $1x10^8$ conidia/ml. While at lower concentrations dead larvae were first recorded at 6 days after treatment. The highest number of larvae died within 10-12 days after treatment for all concentrations. Sufficient mortality resulted when larvae were treated with concentrations of $5x10^7$, $7.5x10^7$ and $1x10^8$ conidia/ml. The calculated LC_{50} was 1.2127 $x10^7$, while LC_{90} was 9.7625 $x10^7$ conidia/ml. at 12 days of treatment.

The calculated LT_{50} values were 12.70, 10.50, 8.60, 8.20 and 7.60 days for the concentrations of $1x10^7$, $2.5x10^7$, $5x10^7$, $7.5x10^7$ and $1x10^8$ conidia/ml, while those of LT_{90} recorded 22.70, 18.20, 13.40, 10.90 and 9.90 days for the same concentrations, respectively.

Table (1): Mortality, LC_{50}, LC_{90}, slope, LT_{50} and LT_{90} of *Ostrinia nubilalis* 3rd larval instar treated with *Beauveria bassiana* under laboratory conditions using dipping method.

Conc. conidia/ml	Days after treatment						LC_{50} at 12 days	LC_{90} at 12 days	Slope value at 12 days	LT_{50}	LT_{90}
	2	4	6	8	10	12					
$1x10^7$	0	0	5	15	25	45	1.2127x10^7 conidia/ml	9.7625 x10^7 conidia/ml	0.76	12.7	22.7
$2.5x10^7$	0	0	10	25	40	65				10.5	18.2
$5x10^7$	0	5	15	50	65	85				8.6	13.4
$7.5x10^7$	0	5	30	50	75	100				8.2	10.9
$1x10^8$	0	15	35	65	100					7.6	9.9
control	0	0	0	0	0	0					

Larvae of *O. nubilalis* were found to be infected by the fungus *B. bassiana* (Andreadis, 1980; Marcandier and Riba, 1986; Fing *et al.,* 1988 and Bing and Lewis, 1991). Also, Agarwal *et al,* (1985) found that the fourth instar larvae of *Pyrausta machaeralis* were killed in 1-3 days after treatment, while

5th and 6th instars showed 50-100% mortality in 2-6 days. Both, *Bt* preparations and *B. bassaina* and *Metarahizum anisopliae* to egg, larval and pupal and adult stages of the Asian corn borer, *Ostrinia furnacalus* gave sufficient mortality in laboratory experiments (Chiuo and Hou, 2009).

Table (2) illustrated percent mortality of *S. cretica* larvae treated with five different concentrations; the first dead larvae were recorded on the 6th day after treatments, and the highest mortality occurred between the 12-14 day. The total larval mortality increased as the conidia concentration increased, it was 30, 40, 70, 75 and 90 % for concentrations of $1x10^7$, $2.5x10^7$, $5x10^7$, $7.5x10^7$ and $1x10^8$ conidia/ml, respectively.

The calculated LC_{50} was $8.537x10^7$, while LC_{90} was $15.07\ x10^7$ conidia/ml. Whereas the calculated LT_{50} values were 17.40, 14.80, 11.00, 10.70 and 8.30 days while those of LT_{90} recorded 39.60, 26.60, 18.00, 17.50 and 13.30 days for different concentrations, respectively.

Larvae of *S. cretica* seemed to be susceptible to *B. bassiana* infection. These results are in agreement with those of different laboratory tests which showed that *B. bassiana* is highly pathogenic to a number of lepidopterous pests, *Zuzera pyrina* (Deseo *et al.,* 1984), *Pieris rapae* (Abo Aiana, 1985 and El-Sufty *et al.,* 1986) *Zeuzera coffeae* (Ultomo *et al.,* 1988).

Table (2): Mortality, LC_{50}, LC_{90}, slope, LT_{50} and LT_{90} of *Sesamia cretica* 3rd larval instar treated with *Beauveria bassiana* under laboratory conditions using dipping method.

Conc. conidia/ml	Days after treatment							LC_{50} at 14 days	LC_{90} at 14 days	Slope value at 14 days	LT_{50}	LT_{90}
	2	4	6	8	10	12	14					
$1x10^7$	0	0	0	5	10	20	30	$8.537\ x10^7$	$15.03\ x10^7$	0.571	17.40	39.60
$2.5x10^7$	0	0	0	5	20	30	40				14.80	26.60
$5x10^7$	0	0	5	20	40	55	70				11.00	18.00
$7.5x10^7$	0	0	5	25	45	55	75				10.70	17.50
$1x10^8$	0	0	15	35	60	90	90				8.30	13.30
control	0	0	0	0	0	0	0					

Table (3) shows mortality of *C. agamemnon* larvae treated with different concentrations of *B. bassiana* conidia. The first dead larvae were recorded on the 6th day after treatment. Mortality increased during the 10-14 days after treatment. The total mortality were; 40, 55, 65, 75 and 90 % for $1x10^7$, $2.5x10^7$, $5x10^7$, $7.5x10^7$ and $1x10^8$ conidia/ml, respectively. However, satisfactory mortality 90 % was obtained at $1x10^8$ conidia/ml. Calculated LC_{50} was $1.5884\ x10^7$ while LC_{90} was $9.8329x10^7$ conidia/ml. Whereas, the calculated LT_{50} values were 15.50, 11.80, 8.80, 8.60 and 8.10 days for concentrations of $1x10^7$, $2.5x10^7$, $5x10^7$, $7.5x10^7$ and $1x10^8$ conidia/ml, respectively.

Topical application and stem injection with African isolates of *B. bassiana* were investigated by Tefera and Pringlers (2003) they found the effect of exposure methods, conidial concentrations and sporulation in the second instar of *Chilo partellus* resulted in high mortality (98-100 %). The longest LT_{50} 3.5 days.

Table (3): Mortality, LC_{50}, LC_{90}, slope, LT_{50} and LT_{90} of *Chilo agamemnon* 3rd larval instar treated with *Beauveria bassiana* under laboratory conditions using dipping method.

Conc. conidia/ml	Days after treatment							LC_{50} at 14 days	LC_{90} at 14 days	Slope value at 14 days	LT_{50}	LT_{90}
	2	4	6	8	10	12	14					
$1x10^7$	0	0	0	15	30	40	40	$1.5884 x10^7$	$9.8329 x10^7$	1.619	15.0	38.00
$2.5x10^7$	0	0	5	25	45	55	55				11.80	22.00
$5x10^7$	0	0	5	40	60	65	65				8.80	13.70
$7.5x10^7$	0	0	5	45	70	75	75				8.90	13.2
$1x10^8$	0	0	15	50	80	90	90				8.10	12.80
control	0	0	0	0	0	0	0					

Based on (LC_{50} & LC_{90}) and (LT_{50} & LT_{90}) results revealed that larvae of *O. nubilalis* were the most susceptible to *B. bassiana* followed by *C. agamemnon* and *S. cretica* with values of ($1.2127 x10^7$, $9.7625x10^7$), ($8.537x10^7$, $15.03x10^7$) and ($1.5884x10^7$, $9.8329x10^7$) for the LC_{50} and LC_{90}, respectively. Also, results obtained cleared that LT_{50} and LT_{90} required for the three larval species paralleled with that of LC_{50} and LC_{90}.

Table (4): Comparative toxicity of *Beauveria bassiana* against the three larval species of corn borers using dipping technique method.

Tested parameters	Larval species		
	Ostrinia nubilalis	***Sesamia cretica***	***Chilo agamemnon***
LC_{50} (c/ml)	$1.2127x10^7$	$8.537x10^7$	$1.5884x10^7$
LC_{90} (c/ml)	$9.7625x10^7$	$15.03x10^7$	$9.8329x10^7$
LT_{50} (in days)	7.6-12.7	8.3-17.4	8.1-15.0
LT_{90} (in days)	9.9-22.7	13.3-39.6	12.8-38.0

Where: C/ml = conidia/ml

In conclusion, the pathogenicity of the fungus *B. bassiana* against larvae of corn borers due to the fungus spore germinate on the cuticle surface and produces an infections hypha, which penetrates the integument of the insect at any point except the head capsule. The penetration appears to be assisted by the production of enzymes that dissolve the chitinous layer of the cuticle and by the mechanical pressure exerted by the fungus. After infection through the cuticle, the fat body is the first tissue to be attacked. The infected larvae become sluggish and furls to respond to external stimuli. The larva remains soft until the mycelium has ramified and grown in the body tissues following this the body becomes rigid and mummified. No external signs of the fungus are evident as long as the larva is kept in a dry atmosphere. Soon after exposure to moist air, the white mycelium becomes apparent over the surface of the insect.

Generally, the results illustrated in this study by using the fungus *B. bassiana* to the three species of corn borers are of great importance and encourage the use of this fungus strain as a microbial insecticide against corn borers these results need further studies to ensure under field conditions at

Kafr El-Sheikh Governorate. The high susceptibility of the larvae to disease infection and suitable weather prevailing in maize and rice fields particularly the high relative humidity under Kafr El-Sheikh conditions in North Delta.

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التأثير الممرض لفطر البيوفاريا باثينا على ثاقبات الذرة تحت الظروف المعملية

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قسم بحوث المكافحة الحيوية- مركز البحوث الزراعية-معهد بحوث وقاية النباتات بسخا-مصر

تعتبر المكافحة الميكروبية للحشرات أحد طرق المكافحة الحيوية التى استخدمت بفاعلية منذ زمن طويل وتعتبر الآن فى دائرة الاهتمام فى ضوء حتمية التقليل من تلوث البيئة ويعتبر فطر البوفاريا باسينا من مسببات أمراض الحشرات التى تصيب وتقتل أنواع كثيرة منها ولقد تم عزل سلالة من هذا الفطر وجدت على يرقات دودة القصب الكبيرة (بكفر الشيخ)، واستخدمت هذه السلالة فى الدراسة المعملية الحالية-ولقد تم عزل وتنمية الفطر على بيئة صناعية داخل أطباق بترى وبلغ متوسط عدد الجراثيم 2.5 x 10^{10}/طبق وتم تجهيز التركيـــزات التاليـــة: 1 × 10^{7} ، 2.5 × 10^{7} ، 5 × 10^{7} ، 7.5 × 10^{7} ، 1 × 10^{8} كونيديا/مل وعوملت يرقات العمر الثالث بطريقة الغمر لمدة 3 ثوان فى محلول الفطر وكانت أهم النتائج المتحصل عليها كالآتى:

1- بالنسبة لثاقبة الذرة الأوربية: كانت نسبة الموت عالية (85-100%) عند تركيز 5 × 10^{7} ، 7.5 × 10^{7} ، 1 × 10^{8} كونيديا/مل (أما الوقت اللازم لقتل نصف اليرقات المعاملة فكان 8.6 ، 8.2 ، 7.6 يوماً عند التركيزات المذكورة على الترتيب).
2- بالنسبة لثاقبة القصب الكبيرة كانت النسبة (75-90 %) عند تركيز 5 × 10^{7} ، 7.5 × 10^{7} ، 1 × 10^{8} كونيديا/مل أما الزمن اللازم لقتل نصف اليرقات المعاملة فكان 11.0 ، 10.7 ، 8.3 يوماً عند التركيزات المذكورة على التوالى.
3- تراوحت نسبة موت يرقات ثاقبة القصب الصغيرة ما بين 80 – 90% عند تركيزات 7.5 × 10^{7} ، 1 × 10^{8} كونيديا/مل أما الوقت اللازم لقتل نصف اليرقات المعاملة فكان 15.0 ، 11.8 ، 8.8 ، 8.6 ، 8.1 عند جميع التركيزات المستخدمة على الترتيب.

وتشير هذه النتائج المعملية إلى إمكانية استخدام هذه التركيزات للفطر بنجاح ضد هذه الآفات فى حقول الذرة والأرز خاصة فى منطقة كفر الشيخ بشمال الدلتا، حيث الظروف الجوية المناسبة من درجات حرارة ورطوبة.

قام بتحكيم البحث

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