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Isolation and Virulence of *Beauveria bassiana* (Bals.) Vuill. (Deuteromycotina: Hyphomycetes) Isolates from Hibernated *Ostrinia nubilalis* (Hb.) and *Sesamia cretica* Led. from Maize Stalks

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ABSTRACT

The experiments were carried out at three locations in Gharbia governorate which heavily infested with corn borers larvae, *Ostrinia nubilalis* and *Sesamia cretica*, on maize stalks abandoned in the field, these locations were Tanta, El-Santa and Quator locations during the period from November to April in 2014/15 season. A proportion reduction in number of the two pests occurred during overwintering in maize stalks by entomopathogenic fungi, *Beauveria bassiana* isolates. The rate of reduction revealed 28.57, 6.74 and 10.41 % of *O. nubilalis* larvae at Tanta, El-Santa and Qutour, resepectively and 7.69 % in *S. cretica* larvae only in El-Santa. Seventeen fungi isolates of the fungus *B. bassiana* were found on *O. nubilalis* larvae at the three locations then the germination of fungi isolates was measured and found that the Bb-OLT 26 Jan isolate was the highest of conidia germinated then selected to bioassy study. The two fungi isolates Bb-OLT 26 and Bb-SLE 26 Jan were treated on *O. nubilalis* and *S. cretica*, respectively in labortatory conditions with concentrations of 10^4, 10^5, 10^6, 10^7 and 10^8 conidia/ml. Therefore the LC_{50} values were 1.23 x 10^5 and 1.18 x 10^7 conidia/ml. for Bb-OLT 26 and Bb-SLE 26 Jan isolates, respectively. This results indicated that the entomopathogenic fungus, *B. bassiana* is an effective a natural pathogen causing a consideral mortality in the hibernated larvae of *O. nubilalis* which may be important in the natural regulation of the pest and encourage to apply it on the field populations at maize fields.

Keywords: *Beauveria bassiana*, *Ostrinia nubilalis , Sesamia cretica,* Maize

INTRODUCTION

Maize is one of the important crop in Egypt. This crop is heavily infested by European corn borer, *Ostrinia nubilalis* (Hb.) (Lepidoptera: Pyraustidae) and Corn stem borer, *Sesamia cretica* Led. (Lepidoptera: Noctuidae). The two pests annually incur drastic loses in maize. Following yield picking, the pests overwinter as full grown larvae inside maize stalks abandoned in the field until next spring (Abd El-Rahman *et al*., 1983 and El-Sherif *et. al*., 1987).

Entomopathogenic microorganisms are important naturally occurring mortality factors of *O. nubilalis* (Phoofolo *et al.* 2001 and Saranraj and Jayaprakash 2017). They comprise bacteria, fungi, microsporidia and nematodes. Fungi as well as bacteria were frequently found as pathogens. During 1995 – 1998, *B. bassiana* strains were isolated from European corn borer, *O. nubilalis* larvae collected in Solovakia (Cagan and Uhlik 1999).

The present work was undertaken to study the role of entomopathogenic fungi isolates as a biological control agents on the population size of the two pests during overwintering. In addition to bioassy of these isolates to benefit from field application.

MATERIALS AND METHODS

1- Estimation of hibernated larval population in maize stalks

The experiment was conducted at three locations in Gharbia governorate which heavily infested with *O. nubilalis* and *S. cretica*, these were Tanta, El-Santa and Quator locations during the period from November to April in 2014/15 season. Maize stalks from one carat were collected from the three locations in November 2014. The stalks were put in the fields at the same enviromental factors of each location. Samples were taken at 15 days interval, where 10 plants were taken from each location. The plants were completely dissected in the laboratory. All obtained larvae of *O. nubilalis* and *S. cretica* (alive and dead) were counted and preserved to isolate of entomopathogenic fungi.

2- Mycosis test

Alive larvae of *O. nubilalis* and *S. cretica* were maintained on filter papers inside sterilized petri dishes to determine the diseased ones. Dead larvae which obtained from stalks and that died in the laboratory were externally cleaned by immersing them in 0.5% sodium hypochorite for 10 seconds and washed in distilled water for 20 seconds. Dead larvae were arranged on filter papers saturated with water inside petri dishes and maintained at 25±2c. After 3-5 days, some cadavered with white homogeneous fungus growth was transferred on new saturated filter papers inside sterilized petri dishes to avoid contamination until conidia formation. Larvae which killed with fungus were counted (El-Sheikh, 2012). The obtained symptoms of the disease were compared with recorded by (Steinhaus, 1949 and Cantwell, 1974). The entomopathogenic fungi isolates were recognized from the Mycology Center, Faculty of science, Assiut University (AUMC) and all isolates were *B. bassiana* isolates which seventeen isolates from *O. nubilalis* larvae and one isolate from *S. cretica* larvae (Table1).

3-Conidia viability

To select the virulence isolate conidia viability were determined at the time of each isolate, concentration of 10^4 conidia/ml. was sprayed onto petri dishes containing PDA. The conidia were incubated for 24 h at 25±2C. After incubation, three droplets of lactophenol cotton blue stain (0.5% cotton blue) were added to each petri dish to fix and stain the conidia, preventing any further germination from occurring in the sample. The droplets were covered with a glass slide and evaluated using microscope. The number of conidia that germinated in the first 100 conidia observed under the microscope. A conidium was considered to be viable if it germinated (the length of the germ tube was visible

and greater than or equal to the width of the conidium). Viability estimates for all treatments were based on the proportion of conidia that had germinated after incubation (Goettle and Inglis, 1997).

Table 1. The entomopathogenic fungus, *B. bassiana* isolated from insect populations occurring in maize stakes during season 2014/15 in three experimental locations at Gharbia governorate.

Fungus species	Isolate abbreviation	Host insect	locations
B. bassiana	Bb-OLT 26 Jan Bb-OLT 10 Feb Bb-OLT 25 Feb Bb-OLT 12 Mar Bb-OLT 27 Mar Bb-OLT 10 Apr	*O. nubilalis* larvae	Tanta
	Bb-OLS 26 Jan Bb-OLS 10 Feb Bb-OLS 25 Feb Bb-OLS 12 Mar Bb-OLS 27 Mar Bb-OLS 10 Apr	*O. nubilalis* larvae	El-Santa
	Bb-OLQ 26 Jan Bb-OLQ 10 Feb Bb-OLQ 12 Mar Bb-OLQ 27 Mar Bb-OLQ 10 Apr	*O. nubilalis* larvae	Quator
B. bassiana	Bb-SLS 26 Jan	*S. cretica* larvae	El-Santa

4- Bioassay

Third and fourth larval instars of *O. nubilalis* and *S. cretica* were collected from infested maize stalks, each borer species was represented by 240 larvae divided into six groups, 40 larvae each. Five groups were treated with different concentrations of isolate suspension containing 0.1% Tween 80; 10^4, 10^5, 10^6, 10^7 and 10^8 conidia/ml. The 6th group was treated with tap water containing 0.1% Tween 80 as a control. Larvae of *O. nubilalis* treated with Bb-OLT 26 Jan isolate while larvae of *S. cretica* treated with Bb-SLE 26 Jan isolate. The larvae were individually treated by dipping in the fungus isolate. Each ten larvae were introduced into a Petri dish having 5 cuts of maize stems about 5cm long for feeding. The larvae were examined every two days and larval mortality was recorded for two weeks.

RESULTS AND DISCUSSION

1- Estimation of hibernated *O. nubilalis* larval population and the incidence of the entomopathogenic fungus, *B. bassiana* isolates in maize stalks

The numder of *O. nubilalis* hibernated larvae in maize plants and the incidence of the entomopathogenic fungus *B. bassiana* isolates were counted from three locations in Gharbia governorate: Tanta, El-Santa and Quator during the period from November to April in 2014/15 season are presented in Table (2).

In maize stalks, *O. nubilalis* hibernated larvae were found from 27th of November to 10th of April at Tanta and El-Santa while at Qutour were found from 27th of December to 10th of April. Population size of larvae reached a peak on 26th of January at Tanta and at El-Santa was 12th Decamber while in Qutour was 25th of Febrewary and 27th of March. The total number of larvae sampled through the season was higher at El-Santa followed by Tanta and Qutour was 178, 56 and 48 larvae/ 10 plants, resepectively.

Table 2. Population size of *O. nubilalis* larvae and the incidence of the entomopathogenic fungus *B. bassiana* isolates, sampled in hibernation period from maize stalks during season 2014/15, at Gharbia governorate.

Sampling date	Tanta			El-Santa			Qutour		
	larvae/ 10 plants	Fungus incidence		larvae/ 10plants	Fungus incidence		larvae/10 plants	Fungus incidence	
	No.	No.	%	No.	No.	%	No.	No.	%
27 Nov.	8	0	0	21	0	0	0	0	0
12 Dec.	6	0	0	37	0	0	0	0	0
27 Dec.	2	0	0	28	0	0	1	0	0
11 Jan.	0	0	0	27	0	0	0	0	0
26 Jan.	16	7	43.75	17	3	17.64	2	1	50
10 Feb.	3	2	66.6	9	1	11.1	6	1	16.6
25 Feb.	3	2	66.6	16	3	18.75	10	0	0
12 Mar.	5	1	20	10	2	20	12	1	8.3
27 Mar.	9	2	22.2	7	2	28.57	10	1	10
10 Apr.	4	2	50	6	1	16.6	7	1	14.28
Population sampled in the season	56	16		178	12		48	5	
Seasonal incidence of fungi %		28.57				6.74			10.41

The incidence of the fungi isolates was first recorded in larval population on 26th of January in the three locations to 10th of April. The peak of the fungus incidence was 66.6% in 10th and 25th of Febrwary at Tanta and was 28.57% in 27th of March at El-Santa while was 16.6% in 10th of Febrwary at Qutour. The seasonal fungal incidence of larvae was higher at Tanta followed by Qutour and El-Santa was 28.57, 10.41 and 6.74 %, resepectively. Also, Cagan and Uhlik (1999) isolated certain strains of *B. bassiana* from dead *O. nubilalis* larvae collected during the autumn in maize plants at various locations in Solovakia.

2- Estimation of hibernated *S. cretica* larvae population and the incidence of the entomopathogenic fungus *B. bassiana* isolates in maize stalks

The numder of *S. cretica* hibernated larvae in maize stalks and the incidence of the entomopathogenic fungus *B. bassiana* isolates were counted from three locations in Gharbia governorate: Tanta, El-Santa and Quator during the period from November to April in 2014/15 season are presented in Table (3).

In maize stalks, *S. cretica* hibernated larvae were found with a few number in all locations. The total number of larvae sampled through the season was higher at El-Santa followed by Qutour and Tanta were 13, 4 and 3 larvae/ 10 plants, resepectively. The incidence of the fungi isolate was only recorded in larval population on 26th of January at El-Santa. The seasonal fungal incidence of larvae at El-Santa was 7.69 %.

Table 3. Population size of *S. cretica* larvae and the incidence of the entomopathogenic fungus *B. bassiana* isolate, sampled in hibernation period from maize stalks during season 2014/15 at, Gharbia governorate.

Sampling date	Tanta			El-Santa			Qutour		
	larvae/ 10 plants	Fungus incidence		larvae/ 10 plants	Fungus incidence		larvae/ 10plants	Fungus incidence	
	No.	No.	%	No.	No.	%	No.	No.	%
27 Nov.	0	0	0	4	0	0	0	0	0
12 Dec.	1	0	0	0	0	0	0	0	0
27 Dec.	0	0	0	3	0	0	2	0	0
11 Jan.	0	0	0	0	0	0	2	0	0
26 Jan.	1	0	0	2	1	50	0	0	0
10 Feb.	0	0	0	0	0	0	0	0	0
25 Feb.	0	0	0	1	0	0	0	0	0
12 March	0	0	0	0	0	0	0	0	0
27 March	0	0	0	2	0	0	0	0	0
10 Apr.	1	0	0	1	0	0	0	0	0
Population sampled in the season	3			13			4		
Seasonal incidence of fungi%			0			7.69			0

5-Conidia viability

Germination of *B. bassiana* isolates from *O. nubilalis* larvae population occurring in maize stakes during season 2014/15 in three experimental locations at Gharbia governorate are presented in Table (4).

The germination of fungi isolates was ranged from 76 to 97 % at Tanta and ranged from 72 to 94 % at El-Santa, while at Qutour ranged from 62 to 86 %. Accordingly, the Bb-OLT 26 Jan isolate was the highest of conidia germinated then selected to additional study.

Table 4. Germination of *B. bassiana* isolates from *O. nubilalis* larvae population occurring in maize stakes during season 2014/15 in three experimental locations at Gharbia governorate.

B. bassiana isolates	Germination %	location
Bb-OLT 26 Jan	97	Tanta
Bb-OLT 10 Feb	81	
Bb-OLT 25 Feb	93	
Bb-OLT 12 Mar	76	
Bb-OLT 27 Mar	87	
Bb-OLT 10 Apr	93	
Bb-OLS 26 Jan	89	El-Santa
Bb-OLS 10 Feb	80	
Bb-OLS 25 Feb	94	
Bb-OLS 12 Mar	79	
Bb-OLS 27 Mar	88	
Bb-OLS 10 Apr	72	
Bb-OLQ 26 Jan	82	Qutour
Bb-OLQ 10 Feb	70	
Bb-OLQ 12 Mar	62	
Bb-OLQ 27 Mar	75	
Bb-OLQ 10 Apr	86	

6-Bioassay

As shown in Table (5) the two fungi isolates: Bb-OLT 26 Jan isolated from *O. nubilalis* larvae and Bb-SLE 26 Jan isolated from *S. cretica* larvae were selected to study their efficiency on third and fourth larval instars of each borer species. For each fungi isolate, conidia suspensions contained 10^4, 10^5, 10^6, 10^7 and 10^8 conidia/ml. All concentrations induced mortality for the insects and mortality increased as the conidia concentration increased. The mortality of insects began throughout the period extending from the 4th to the 14th days after treatment for the Bb-SLE 26 Jan isolate while was from the 6th to the 14th days after treatment for the Bb-SLE 26 Jan isolate. In Bb-OLT 26 Jan isolate corrected mortality ranged 31.58 – 89.47 % while in Bb-SLE 26 Jan isolate was ranged 17.5 – 67.5 %. Results agree with those of (Cagan and Uhlik 1999) who tested *B. bassiana* strains isolated from *O. nubilalis* against larvae of *O. nubilalis* in labortory conditions(25ºc) and they observed dead larvae of *O. nubilalis* after 48 hours from the application and after seven days, the mortality reached 87%.

Data represented in Table (6) showed that the calculated LC_{50} values were 1.23×10^5 and 1.18×10^7 conidia/ml. for Bb- OLT 26 Jan and Bb-SLE 26 Jan isolates, respectively and values of slopes. The probit regression lines presented in fig. 1. This results indicated that the *B. bassiana* isolated from *O. nubilalis* larvae is more virulent than *B. bassiana* isolated from *S. cretica* larvae. In similar study, Mansour (1999) evaluated the efficacy of the fungus, *B. bassiana* against *O. nubilalis* in the laboratory. He treated the larvae with suspensions of $1x10^7$, $2.5x10^7$, $5x10^7$, $7.5x10^7$ and

$1x10^8$ conidia/ml and obtained mortality ranged 40-90%. Also, Sewify (1999) in Egypt studied the pathogenicity of three *B. bassiana* isolates against corn pests. He used the most virulent isolate in a field experiment to evaluate the efficacy of the fungus against the lepidopterous insects, *Ostrina nubilalis*; *Cryptoblabes gnidiella* Mill and *Chilo agamemnon* Bles. He obtained a major increase in corn yield after the treatment.

Table 5. Mortality of *O. nubilalis* and *S. cretica* larvae treated with different conidia concentrations of *B. bassiana* Bb-OLT 26 Jan and Bb-SLE 26 Jan isolates, respectively under laboratory conditions.

Fungal isolates	Concentration Conidia/ml	Mean No. of dead larvae (days after treatment)							Mortality	
		2	4	6	8	10	12	14	%	corrected
Bb-OLT 26 Jan	1×10^4	0	0	1	6	11	13	14	35	31.58
	1×10^5	0	0	2	6	13	16	19	47.5	44.74
	1×10^6	0	2	6	12	22	26	29	72.5	71.05
	1×10^7	0	2	7	15	27	30	32	80	78.95
	1×10^8	0	4	11	22	31	35	36	90	89.47
Bb-SLE 26 Jan	1×10^4	0	0	0	1	3	6	7	17.5	17.5
	1×10^5	0	0	0	1	4	6	9	22.5	22.5
	1×10^6	0	0	0	2	5	7	9	22.5	22.5
	1×10^7	0	0	1	5	8	14	21	52.5	52.5
	1×10^8	0	0	2	6	11	22	27	67.5	67.5

The obtained results are confirmed by Yasin *et al.* (1999) in Indonesia, who investigated the effectiveness of *B. bassiana* for controlling *Ostrinia furnacalis* Guen. larvae. They used six concentrations: 5 x 10^7, 5 x 10^6, 5 x 10^5, 5 x 10^4, 5 x 10^3 conidial/ml, and control. Results indicated that the effectiveness of fungus began at three days after inoculation and the concentrations 5 x 10^5 conidial/ml or above were effective to control *O. furnacalis* larvae. Also, Sabbour (2002) in Egypt investigated three bioinsecticides derived from *Bacillus thuringiensis*, *Beauveria bassiana* and *Verticillium lecanii* against three corn borer pests *Sesamia cretica* Led., *Chilo agamemnon* Bles. and *Ostrinia nubilalis*. In the field trials, *B. bassiana* gave the best results, followed by *V. lecanii* and *B.thuringiensis*.

Table 6. Values of LC_{50} (conidia/ml) for the two isolates of the entomopathogenic fungi against *O. nubilalis* and *S. cretica* larvae.

Fungal isolates	LC_{50} value conidia/ml.	Lower	Upper	Slop
Bb-OLT 26 Jan	1.23×10^5	0.51×10^5	2.93×10^5	0.4104
Bb-SLE 26 Jan	1.18×10^7	4.63×10^6	3×10^7	0.3399

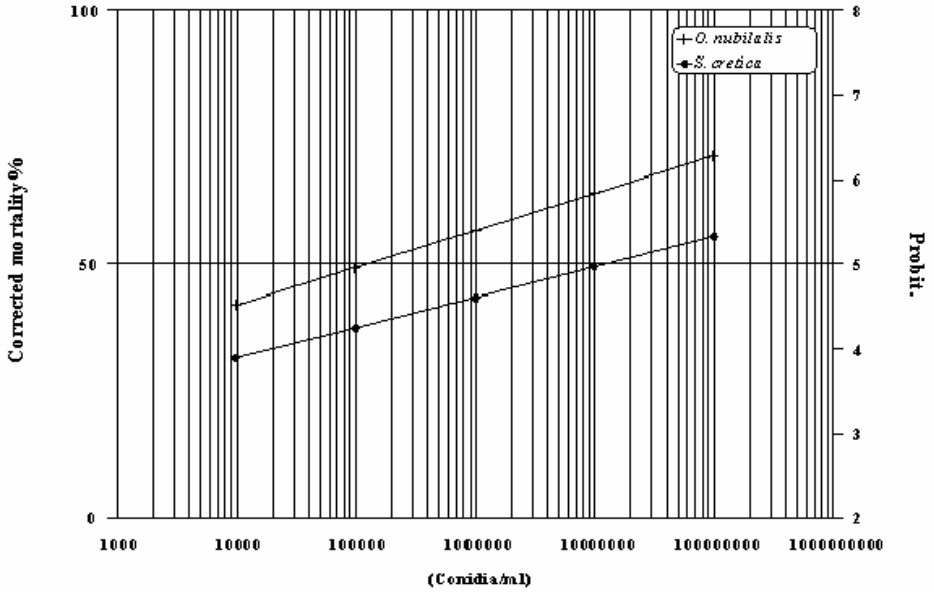

Fig 1. Mortality response of *O. nubilalis* and *S. cretica* larvae to different concentrations of Bb-OLT 26 Jan and Bb-SLE 26 Jan fungi isolates, respectively.

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عزل وتقدير فاعلية العزلات الفطرية الممرضة للحشرات *Beauveria bassiana* (Bals.) Vuill. (الفطريات الناقصة: الفطريات الهيفية) المعزولة من يرقات كلا من حفار ساق الذرة الأوروبي و دودة القصب الكبيرة أثناء البيات الشتوي بسيقان الذرة

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أجريت هذه الدراسة في ثلاث مناطق بمحافظة الغربية والتي تميزت بنسبة إصابة عالية من يرقات ثاقبات الذرة: حفار ساق الذرة الأوربي و دودة القصب الكبيرة في سيقان الذرة المخزنة في الحقل وهذه المناطق هي طنطا، السنطة و قطور في موسم 2014//15. وجد قدر من الانخفاض في عدد هاتان الآفتان أثناء البيات الشتوي بسيقان الذرة بواسطة عزلات من الفطر الممرض الحشري *Beauveria bassiana*. وكان معدل الخفض في يرقات حفار ساق الذرة الأوربي 28.57، 6.74 و 10.41 % في طنطا والسنطة وقطور علي التوالي وكان 7.69% ليرقات دودة القصب الكبيرة في السنطة فقط. وتم عزل 17 عزلة فطرية من فطر *B. bassiana* علي يرقات حفار ساق الذرة الأوربي وبعد ذلك تم قياس إنبات العزلات الفطرية ووجد ان العزلة Bb-OLT 26 Jan كانت الاعلي في نسبة إنبات الجراثيم وتم اختيارها لدراسة التقييم الحيوي. تم معاملة العزلتين الفطريتان Bb-OLT 26 Jan و Bb-SLE 26 Jan علي يرقات حفار ساق الذرة الأوربي و دودة القصب الكبيرة علي التوالي تحت الظروف المعملية بتركيزات 10^4, 10^5, 10^6, 10^7, 10^8 جرثومة/مللتر. ومن ثم كانت قيم التركيز المميت لنصف العشيرة هو 1.23×10^5 و 1.18×10^7 جرثومة/مللتر لكل من العزلتين الفطريتان Bb-OLT 26 Jan و Bb-SLE 26 Jan علي التوالي. هذه النتائج توضح أن الفطر الممرض الحشري *B. bassiana* هو ممرض حيوي طبيعي فعال يسبب نسبة موت ملحوظة ليرقات حفار ساق الذرة الأوربي التي تبيت شتويا والذي يكون مهم في التوازن الطبيعي للآفة والذي يشجع تطبيقه حقليا علي عشيرة الحشرة بحقول الذرة.