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EVALUATION STUDIES OF SOME BIO-CONTROL AGENTS AGAINST CORN BORERS IN EGYPT

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ABSTRACT

Maize (corn) (*Zea mays* L.) is infested by three corn borer species namely *Sesamia cretica, Chilo agamemnon* and *Ostrinia nubilalis.* The bioeffect of three bio-insecticides derived from *Bacillus thuringiensis, Beauveria bassiana* and *Verticillium lecanii* and three Chloroformic plant extract from *Melaleuca ericifolia* and *Melaleuca leucadendron* leaves beside a compound derived from *M. ericifolia* leaves (Ursolic acid) were tested against the three corn borer pests. The fungus *B. bassiana* significantly affected the best results, followed by *V. lecanii* and *B. thuringiensis* against the corn borer infestation in the field. The tested botanicals showed nearly similar effects on the target insects. Though they achieved lesser effects than those obtained by microbial formulations, they revealed significant reduction in infestation as compared with the corresponding controls. Yield loss was significantly decreased when maize plants were treated with *B. bassiana, V. lecanii, B. thuringiensis, M. leucadendron, M. ericifolia* and Ursolic acid, respectively.

Key words: *Bacillus thuringiensis, Beauveria bassiana, Verticillium lecanii, Melaleuca ericifolia, Melaleuca leucadendron* and Ursolic acid.

INTRODUCTION

The greasy cutworm *Sesamia cretica* (Lepidoptera- Noctuidae), the lesser sugar cane, *Chilo agamemnon* (Lepidoptera-Pyralidae), and the European corn borer *Ostrinia nubilalis* (Lepidoptera- Pyralidae) are the most destructive corn borers in Egypt and other countries **(Harris, 1985 and 1989) and Othman (1996).**

Attempts are now being made to derive integrated management approach through agricultural and biological control tactics.

Accordingly number of bacterial pathogens as *B. thuringiensis* and *B. cercus* have been isolated from diseased larvae of *Chilo spp. S. cretica* and *O. nubilalis* **(Varama and Singh 1987 and Ansari *et al* 1987).** Now the entomopathogens, *B. thuringiensis* and *B. bassiana* are widely advocated in many IPM. Programs. However, several spices of transgenic plants including cotton, potatoes and tomatoes have now been developed that produce *B.t*-endotoxin as synthetic insecticides **(Perlek *et al* 1990).**

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Many insect pests can be controlled by plant extracts and by some phenols (**Mansour *et al* 1993**). **Jill (1993)** suggested that the higher plants can used as pesticides for many insects. **Marcos Kogan (1986)**, found that natural chemicals in plant tissues can control some insect pests. **Arnason *et al* (1987)** reported that limonoids extract reduce the feeding growth and developments of the *O. nubilalis*.

The present work was aimed to evaluate the bioeffect of bioinsecticides *B. thuringiensis*, *B. bassiana* and *V. lecanii* and three plant extract derivatives against corn borer in Egypt.

MATERIAL AND METHODS

Source and rearing colonies of target insects

Samples of hibernated *Ostrinia nubilalis* (Hubn.) and *Chilo agamemnon* (Hubn.), infested corn and rice stalks were collected from El-Sharkia and El-Gharbia. The hibernating larvae were collected, put in a carton tunnels and kept in Petri dishes (14×2.5cm) under laboratory conditions 26±2°C and 60-70% R.H.

The emerged moths were collected, sexed and kept in two liter cylindrical glass jars (3 females and 3 males each jars). The inner surfaces of the jars were lined with waxy paper for oviposition. The emerged moths were provided with small cotton pieces soaked in 10% honey solution for feeding strips of waxy paper carrying eggs masses (20-25 eggs), each were cut and kept tightly in a glass jar with 3gm of semi-artificial diets (**Salama, 1970**). The diet consists of dry kidney beans powder 50 gm, brewer yeast 16gm, benzoic acid 2 gm, formaldehyde 1 ml, 0.1 ml Hcl / ml agar 9 gm water up to 500 ml.

For *Sesamia cretica* the infested stem parts, carrying deposited eggs, were cut in pieces and transferred to aerated glass specimen tube (20×5 cm) provided with moisten cotton tuft to maintain the inside at 100% R.H. The larvae were reared on semiartificial diet (**El-Metwaly *et al* 1997**). The diet components were 450 ml distilled water, 9 gm agar, 18gm yeast extract, 40gm corn leaf powder, 20-25gm Senn Flour, 9 gm glucose, 9gm casein, 1.35 gm ascorbic acid, 4.5ml sorbic acid10% and4.5ml paraben 20%.

Bio-effect of the experimented biocides

The commercial Diple-2x compound based on *Bacillus thuringiensis* var *Kurstaki* stereotype 3a, 3b (32.000 I.U/ mg) was tested. Suspensions in water were added to semiartificial diets at the rate of 500, 250, 125, 63, 32 and 16 ug *Bt*/ml diet. The treated larvae were reared on semi-artificial diet. Mortalities were estimated after 7 days. The experiments were replicated 4 times at 26± 2° C and 60-70 % RH. Mortalities percentage were counted and corrected according to **Abbott (1925)**, while LC50's were calculated through probit analysis (**Finney, 1964**).

The fungi (*B. bassiana* and *V. lecanii)* spores were isolated from dead and/or infected larvae and pupae of corn borers, and stored individually in tightly closed sterilized vials at 4°C in a refrigerator. Refrigerated individuals were examined through 24-48 h from the time of storage. Then dipping in 2% sodium hypochlorite for 3-5 min followed by washing with sterile distilled water. Isolates were subcultured on nutrient PDA medium. Isolates were identified at N.R.C. Microbiology Department. The spores of *B. bassiana* and *V. lecanii* were collected from the surface of mycelium growth. The original culture of *B. bassiana* was, $16x10^8$ conidia / ml, the original culture of *V. l* was $8x10^8$ conidia /ml. For *B. bassiana* or *V. lecanii*, 0.5 suspended in 100 ml of distilled water and added 2 drops of emulsifying agents (Tween-80). This gave rise to a concentration of, $16x10^7$ or $8x10^7$ conidia / ml, respectively. The suspension for each pathogen undergo 1-2 fold dilutions, 6 times.

Plant extracts and Methods of extraction

Melaleuca ericifolia leaves and *M. leucadendron* leaves were collected from zoo garden. The plant leaves were air dried at 27± 3° C and ground to fine powder.

Chemical compounds of *M. ericifolia* and *M. leucadendron* were extracted with petroleum ether followed by chloroform. Two grams of dried Chloroformic extract were boiled with charcool. The filtrated extract was evaporated to dryness. Preparative thin layer chromatography was used for isolation of triterpenoides using the solvent system toluene-ethyl-acttane (120:40: 0.5%). Ursolic acid was then extracted from *M. ericifolia* plant. The dried products were re-dissolved in distilled water with 2 drops of tween-80 as wetting agents to prepare 5% suspension for each type.

Laboratory experiments

The target insects spices 25 second larval instars were fed on the selected artificial diet, contaminated with *B. thuringiensis* at the rate of 500, 250, 125, 63, 32, and 16 ug/ml.

For *B. bassiana* added at the concentration of 16, 8, 4, 2, 1, $0.5x10^7$ conidia / ml to the insect diet.

V. lecanii added at the rate of 8, 4, 2, 1, 0.5, 0.25 x 10^7 conidia / ml to the insect diet. Mortality were counted after 7 days, and corrected according to **Abbott, 1925.** LC50, variance and 95% confidence limits were calculated according to **Finney, 1964.**

The plant extracts were added to the diet w/v (5gm of plant extract to 100 ml of distilled water). Counts of mortalities were undertaken after 7days.

All experiments were carried out under laboratory conditions at 26 ±2°C and 60-70% RH. Each experiment was replicated four times.

Field trails

The field trails were carried out during two successive seasons (2000 and 2001) at El-Kanater Agricultural Research Farm attached to the National Research Center (N.R.C), to study the effectiveness of (*B. thuringiensis, B. bassiana, V. lecanii, M. ericifolia, M. leucadendron* and Ursolic acid) under field conditions against the corn borer infestations.

Maize (*Zea mays* L.)Var (Giza 2) was planted at 4 th week of May (during the two seasons) in an area about 2800 m² divided into 28 plots 100 m² each. Each of the tested formulations was then applied as a single treatment in a randomized plot.

Four plots were treated by water as check.

1. *B. thuringiensis*, *B. bassiana* and *V. lecanii* were applied at the rate 20gm/5L water/10m².
2. The plant extract *M. ericifolia*, *M. leucadendron* and Ursolic acid at the rate of 10% was tested.

Each treatment weekly sprayed figures and replicated 4 times.

The infestation of *S. cretica*, *O. nubilalis* and *C. agamemnon* after 20, 50 and 90 days of treatments were estimated. The yield weight averages were determined at harvest season.

Yield assessment

Data presented yield weight figures in kgs for the treated and untreated plots. Yield loss was calculated according to the following equation:

$$\textit{Yield loss} = \frac{\textit{Potential yield} - \textit{actual yield}}{\textit{Potential yield}} \times 100$$

Potential yield was the yield treated with *B. bassiana*.

RESULTS AND DISCUSSION

Evaluating the bio-effect of plant extracts under laboratory conditions

Data in Table (1) show that *M. leucadendron* extract at 5% concentration caused 74, 65 and 59% mortalities among second larval instar of *O. nubilalis*, *S. cretica* and *C. agamemnon*, respectively. The corresponding mortality percentage reached 64, 56 and 59% after *M. ericifolia* treatment and 67, 55 and 50% when treated with Ursolic acid were obtained for these insects, as compared to 0, 1 and 0% in the control, respectively (Table 1). In this concern **Mansour *et al* (1993)**, controlled *O. nubilalis* by some extracted phenols. **Marcos Kogan, 1986** suggested that many plants contain natural chemicals can control the insect pests. In this manner, **Arnason *et al*** (1987) extracted limonoids which can reduce the feeding and development of the European corn borer. **Jill, (1993)**, the higher plants can be used against many insects as pesticides. Many authors controlled the insect pests by plant extracts, **Nassar *et al* (1999) and Mona Magd-El-Din (2001).**

The bio-activity of certain microbial agents against the corn borer insects (second instar larvae).

Data in Table (2) show the LC50's of the pathogens against the target insects.

The LC50's for *B.t* were 136, 141 and 151 ug/ml when second instar larvae of *O. nubilalis*, *S. cretica* and *C. agamemnon* were tested, respectively. Similar results obtained by **(Othman, 1996; Mohamed, 1997 and Abdel-Rahman, 1992)** and recorded that *C. agamemnon* and *O. nubilalis* are susceptible to *B. thuringiensis*, var. *Kurstaki; Entomocidus* and *Aizawai*.

The corresponding LC50's for the fungus *B. bassiana* values were 3.2, 2.0 and 1.1×10^7 conidia/ml, respectively.

O. nubilalis was more tolerant to *V. lecanii* fungus its LC50 reached 2.5×10^7 conidia/ml, while the other insects proved almost similar susceptibilities to both fungi Table (2). The results are in accordance with the results obtained by, **(Othman, 1996; Mansour, 1999 and Abdel-Gawad, 2000)** found that the larval stages of the corn borer were highly susceptible to both fungal Spp.

Table 1. The changes of bio-effect of some plant extracts as indicated by laboratory experiments.

Plant extracts	Insect	Mortality % (mean)
	O. nubilalis	74
M. leucadendron	*S. cretica*	65
	C. agamemnon	59
	O. nubilalis	64
M. ericifolia	*S. cretica*	56
	C. agamemnon	59
	O. nubilalis	67
Ursolic acid	*S. cretica*	55
	C. agamemnon	50
	O nubilalis.	0
Control (untreated)	*S cretica.*	1
	C. agamemnon	0

Table 2. The bio-effect of certain microbial control agents against corn-borer infestations as determined by laboratory experiments..

Pathogen	Insect	LC50	Slope	Variance	95% confidence limits
	O. nubilalis	136	0.6	1.4	122-149
B. thuringiensis	*S. cretica*	141	1.5	2.2	130-157
	C. agamemnon	151	1.4	2.3	143-168
	O. nubilalis	$3.2X10^7$	0.5	2.5	$2.4\text{-}4.4x0^7$
B. bassiana	*S. cretica*	$2.0X10^7$	2.4	1.5	$1.0\text{-}3.2x0^7$
	C. agamemnon		1.4	1.8	
		$1.1X10^7$			$0.2\text{-}2.2x10^7$
	O. nubilalis	$2.5X10^7$	0.3	3.4	$1.4\text{-}4.2x10^7$
V. lecanii	*S. cretica*	$1.7X10^7$	1.3	1.5	$0.4\text{-}2.9x10^7$
	C. agamemnon		1.3	2.7	
		$1.3X10^7$			$0.5\text{-}2.5x10^7$

Moralities corrected according to **Abbott, formula, 1925.**

Field trials

Data in Table (3) summarize the percentage of infestation after treatments with the tested bioinsecticides. The fungi especially, *B. bassiana* showed a high potential effect against corn borer infestations.

Table 3. The changes in the filed trails assessments of damage figures caused after treatments.

Post 1st application date	Treatments*	% of infestation (mean)					
		SEASON 2000			SEASON 2001		
		S. cretica			*S. cretica*		
20	Control	51^{a}			44^{a}		
	B.t	30^{d}			26^{bc}		
	B.b	25^{e}			14^{d}		
	V.l	31^{cd}			23^{c}		
	M. leucadendron	34^{c}			31^{bc}		
	M. ericifolia	39^{b}			34^{b}		
	Ursolic acid	30^{d}			30^{bc}		
	F value	20.03**			11.65**		
	LSD5%	3.12			8.90		
		S. cretica	*O. nubilalis*	*C. agamemnon*	*S. cretica*	*O. nubilalis*	*C. agamemnon*
50	Control	47^{a}	40^{a}	45^{a}	46^{a}	47^{a}	40^{a}
	B.t	26^{e}	26^{d}	28^{e}	27^{d}	24^{c}	29^{c}
	B.b	19^{g}	18^{f}	17^{g}	12^{f}	10^{d}	16^{e}
	V.l	23^{f}	23^{e}	20^{f}	21^{c}	23^{c}	26^{d}
	M. leucadendron	32^{c}	30^{b}	30^{d}	33^{cd}	30^{bc}	30^{c}
	M. ericifolia	30^{d}	34^{a}	32^{c}	38^{b}	31^{b}	31^{bc}
	Ursolic acid	34^{b}	27^{c}	34^{b}	34^{c}	31^{b}	33^{b}
	F value	12.02**	11.56**	10.32*	9.67**	13.08**	14.8**
	LSD5%	1.66	2.31	1.39	1.37	2.09	2.13
90	Control	39^{a}	39^{a}	43^{a}	37^{a}	43^{a}	33^{a}
	B.t	25^{c}	23^{c}	29^{c}	22^{e}	31^{d}	12 c
	B.b	9^{e}	10^{d}	16^{e}	10^{f}	20^{f}	8^{e}
	V.l	20^{d}	25^{c}	22^{d}	25^{d}	28^{e}	11^{d}
	M .leucadendron	31^{b}	33^{b}	30^{c}	33^{b}	37^{b}	24^{b}
	M ericifolia	30^{b}	32^{b}	35^{b}	30^{cd}	34^{c}	23^{bc}
	Ursolic acid	30^{b}	33^{b}	33^{bc}	31^{c}	33^{cd}	26^{b}
	F value	15.10**	12.20**	16.04**	9.89**	10.43**	11.54**
	LSD5%	1.22	2.04	3.12	1.02	2.33	3.44

Values in column with the same letters are not significantly different at 5%levels probability.
**B.t. : Bacillus thuringiensis*
B.b : *Beauveria bassiana*
V.l. : *Verticillium lecanii*

The data show that during season 2000, after 20 days 1st post applications, infestation of *S. cretica* were 51% in the control. In case of *B. bassiana* treated plots this percent was significantly decreased to 25% (Table 3). After application with *B.t., V.l., M. leucadendron, M. ericifolia* and Urisolic acid, the percentage of infection significantly decreased to 30. 30, 34, 39 and 30%, respectively.

After 50 days of the post first application the percentage of infestation by *O. nubilalis* and *C. agamemnon* in the control plots increased to 40 and 45%, respectively. In all bioinsecticides treated areas, the percentages of infestation were significantly decreased, as compared to the corresponding control. In case of *B. bassiana* treated areas, infestation reached 18, 19 and 17% for *O. nubilalis, S. cretica* and *C. agamemnon*, respectively. The percentage of infestation of the target insects decreased to 23, 23 and 20% after treatment by *V.l.* In general, the data show that *B. bassiana* proved the most protective agent among the tested materials for all the target insect pests.

After 90 days of the first post application, also the percentage of infestation by the target insects was significantly decreased after the bioinsecticides applications as compared to the corresponding control in the field.

During season 2001, the same results obtained. Twenty days of the first post application the percentage of infestation with *S. cretica* were significantly decreased after treated by with *B.t, B. b, V. l, M. leucadendron, M. ericifolia* and ursolic acid, 26, 14, 23, 31, 34 and 30% respectively, as compared to 44% in the control.

After 50 days of the post applications by the bioinsecticides tested, the rate of infestation by *O. nubilalis, S. cretica* and *C. agamemnon* were still significantly less than the corresponding controls, respectively.

After 90 days of the applied bioinsecticides the infestation percentage of *O. nubilalis, S. cretica* and *C. agamemnon* were significantly decreased according to the different treatments. **Lewis and Bing (1990)** controlled *O. nubilalis* by *B.t* and *B.b*

Our results agree with (**Hussey and Tinsley, 1981 and Riba 1984**) who found that *Beauveria bassiana* and *Verticillium lecanii* are currently being developed as a mycocoinsecticides for the European corn borer.

In this concern, **Othman, 1996**, isolated *V. lecanii* from *O. nubilalis* in Egypt and found that *S. cretica* was susceptible to the fungal infection. The entomopathogenic fungi *B. bassiana* and *V. lecanii* can protect the plant from the infestation by the target insects for a long time.

Abd-El-Gawad, 2000, suggested that fungal treatments with *B. bassiana* and *V. lecanii* reduce the maize plant infested with corn borer. **Bing and Lewis (1991) and 1992**, found that *B. bassiana* could control *O. nubilalis* in the field.

Foschi and Grassi (1985), found that *B. bassiana* and *M. anisopliae* significantly reduced the number of borer holes per plant. Also *B. bassiana* remained effective for longer time in the field.

Bing and Lewis, (1991 and 1992) found that foliar application of the fungus *B. bassiana* on maize plant caused 98.3% reduction in *O. nubilalis* infestation.

The results agree with **Mansour, (1999)** reported that *B. bassiana* caused a higher mortalities against *O. nubilalis* and *C. agamemnon* than *S. cretica*.

Abdel-Gawad. (2000) found that larval instar of *S. cretica* was highly susceptible to *B. bassiana* followed by *Nomuraea rileyi* and *V. lecanii*. He found also that field application of the entomopathogenic fungi and chemical insecticide against *O. nubilalis, C. agamemnon* and *S. exigua* caused a significant decline in larval population. Also he showed that fungal treatments reduced the number of larvae in maize plants and newly hatched larvae were highly affected by fungal treatments.

Ismail, 1998, suggested that the plant extract of lemon grass leaves powder and *B. thuringiensis* may have a residual activity against *S. cretica* larvae for at least 4 weeks after applications.

Yield assessments

Data in Table (4) show that the weight of ears, recorded highly amount 220±5.09 kgms/fedan after treating the crop by (B.b) as compared with 115±5.98 kgms/fedan in the control plots. The weight of ears in all tested treatments ranged between 199 ±4.40 and 220±5.09 kg / fed. This led to a significantly decrease in reduction of the yield ranged between 4.19-17.59 kg/fed. during season 2000 Table (4). During season 2001 the ears weight significantly increased after treatment with the tested bioinsecticides as compared to 103±4.25 kg/fed in the untreated plots. The percent of reduction in the yield ranged between 4.04- 19.57% as compared to 53.44% in the control Table (4).

The results agree with **Abdel-Gawad (2000)** who found that the treatments with the fungi caused a higher reduction in infestation with the corn borers. He also found that the maize gain yield was significantly increased as a result of the fungal application with *B. b* or *V. l.* In this concern **Semeada, 1998; Sweify, 1998 and Mansour, 1999,** who recorded that the maize yield increased after fungal treatments.

Table 4. Assessments of damage caused after treatments.

	Season 2000		Season 2001	
Treatments	Wt. of ears Kg/ fedan	%of yield loss kg/fedan	Wt. of ears	%of yield loss kg/faden
Control	115±5.98 [d]	47.78	103±4.25 [e]	53.44
B. t	211±9.10 [a]	4.19	213±6.53 [a]	4.04
B.b	220±5.09 [a]	0	222±2.56 [a]	0
V.l	199±4.40 [b]	9.30	201±7.8 [b]	9.56
M.leucadendron	184±5.63 [c]	16.23	188±5.45 [cd]	15.29
M. ericifolia	182±4.80 [c]	17.13	178±5.79 [d]	19.57
Ursolic acid	181± 6.43 [c]	17.59	180±5.40 [c]	19.01
F value	27.949**		20.719**	
LSD5%	10.49		11.87	

It could be concluded that the fungus *B. bassiana* proved to be the most protective agent among the tested materials for all the target insect species, this is followed in descending order, by *B.t*, *V.1* and botanical extracts and / or derivatives.

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دراسات لتقييم بعض المركبات البيولوجية ضد ثاقبات الذرة في مصر

[٦٥]

ماجدة صبور[١]

١- قسم آفات ووقاية النبات – المركز القومي للبحوث – الدقي – القاهرة – مصر

يصاب محصول الذرة بثلاثة أنواع من الثاقبات في مصر وهي دودة القصب الكبيرة و دودة القصب الصغيرة و دودة الذرة الأوربية.

لقد تم تقييم ثلاثة أنواع من المركبات الميكروبية لمقاومة هذه الحشرات وهي *Bacillus thuringiensis, Beauveria bassiana & Verticillium lecanii* ، وثلاثة أنواع من المستخلصات النباتية في المعمل والحقل.

وقد دلت النتائج على أن الفطر من نوع بوفاريا باسيانا هو الأكثر تأثيراً على ثاقبات الذرة في الحقل ، ثم كلا من الفطر *Verticillium lecanii* ، ثم البكتريا *B. thuringiensis* ، ثم المستخلصات النباتية المستخدمة.

وأيضا دلت النتائج على أن كمية فقد المحصول قد قلت بعد المعاملة بالفطر *B. bassiana* ، ثم *V. lecanii* ، ثم النبات المعامل بالبكتريا *B. thuringiensis* بالترتيب أيضا. وقد قلت نسبة الفقد لمحصول الذرة بعد معاملته بالمستخلصات *M. leucadendron, M. ericifolia* and *Ursolic acid*.

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