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Evaluation of some commercial formuilationsagainst*Spodopteralittoralis* and *Hyperabrunneippennis* larvae.

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Abstract

The insecticidal activity of three commercial Bt – formulations and one fungus; namelyDipelDf , W- Bus andProtecto (*Bacillus thuringiensisvar.kurstaki*) and Biofly (*Beauveriabassiana*) were tested against 2nd and 4th instar larvae of *Spodopteralittoralis*(Boisd) and *Hyperabrunneipennis*(Boheman) were studied under laboratory conditions.Results revealed that Bt- formulations caused the larval mortality after treatment of *S. littoralis*2nd and 4th larval instars ranged from 40 to 100 % and 32.5 to 92.5 % and reached 100 ,85 and 100 % for *H.brunneipennis*,respectively at the highest concentration after 7 days of treatment compared to 77.5 % and 60 % for*S.littoralis* and 95 and 85 % for *H.brunneipennis*treatments byBiofly. The DipelDf and W-Bus were highly efficient on the insect larvae, followed by Biofly and Protecto, respectively. Based on the LC_{50}values,DipelDfwas the highest toxic to*S.littoralis*and *H.brunneipennis* than that of the other compounds. Pupation and adults emergence percentages were reduced by all treatments compared to control.

Key words:*Spodopteralittoralis* , *Hyperabrunneippennis*, commercial products , efficiency

Introduction

Among chewing insect pests,*Spodopteralittoralis*(Boisd) (Lepidoptera: Noctuidae) is considered as an important sporadic pest in the world. It causes 25-100 % economic loss **(Dhir*et al* ., 1992;Prayogo*et al*., 2005)** in crops based on crop stage and its population level in the field. The Egyptian alfalfa weevil (EAW),*Hyperabrunneipennis*(Boheman) (Coleoptera :Curculionidae) is considered to be the most serious and destructive pest of alfalfa in Egypt **(Al-Doghairi and Elhag, 2003)**. One annual generation is recorded in Egypt for the EAW (**Hammad*et al.,* 1967**). The larval stage is the most damaging during the weevil life cycle. By feeding on the alfalfa plant's growing tips, the larvae cause skeletonization of leaves, stunting, reduced plant growth, and ultimate reduction in yield. The adults are also, foliar feeders, causing additional, but less significant, damage.

The widespread and intensive use of different synthetic insecticides for controlling this pest increased environmental problems such as insect resistance, excessive persistence of residues, human health hazards and harmful effect on the non- target organisms. From this point of view, it is necessary to minimize the application of pesticides that considered as a main source of environmental pollution and use other compounds may proof as good alternatives of insecticides. In recent years, crop protection based on biological control of crop pests with microbial pathogens as virus, bacteria, fungi and nematodes were considered as valuable tools in pest management **(Bhattacharya *et al.,* 2003).** Entomopathogenic fungi may proof, also, as valuable and play an important role in integrated pest management programs. **(El- Hawary and Abd El-Salam, 2009)**reported thatfungal biological control agents have demonstrated efficacy against a wide range of insect pests including *S.litura* . Successful use of fungal pathogens in pest control depends on selection of right virulent fungal strain formulated in proper way and applied at an appropriate dose against susceptible host stage under favorable environmental conditions**(Asi*et al.,* 2012).**Among the entomopathogenic agents, also, the most widely used biopesticides are subspecies and strains of *Bacillus thuringensis* (Bt). *B. thuringensis* is a spore- forming bacterium well- known for its insecticidal properties due to its ability to produce crystal inclusions during sporulation. Each strain of this bacterium specifically kills one or a few related species of insect larvae such as Lepidopteran, Dipteran and Coleopteran**(Haggag, 2013)**. Commercial Bt products, generally, consist of a mixture of spores and crystals, produced in large fermenters and applied as foliar sprays, much like synthetic insecticides **(Sanchis*et al.,* 1999).** It is known that most Bt formulations have a very short residual activity.The persistence of Bt. spores show an obvious reduction after few days of exposure to weather, and reduction in its viability was progressively correlated with the time elapsed after exposure in the field. The pathogen is not mobile and cannot escape under the unfavorable conditions**.(Mohamed *et al.,* 2010)** .

In the present experiments, the effectiveness of several bioinsecticides against the cotton leafworm*S.littoralis* and alfalfa weevil,*H.brunneipennis* was determined with the intention to find out the best compounds for controlling these economic pests in an integrated pest management program.

Material and Methods

Tested insects:

A- *S.littoralis*

The cotton leafworm larvae of *S.littoralis* were obtained from Agricultural Research Centre, Cairo, Egypt, and were reared on fresh leaves of caster bean (*Ricinuscommunis*) under laboratory conditions of 25±2C° and65± 5 % R.H..**(Adham*et al.*, 2009 and Kamel*et al.*, 2010)**. As larvae reached the 2nd and 4th instars, they were used in the experiments.

B- *H.brunneipennis*

Alfalfa weevil larvae were collected, early in the morning, by using an insect sweepnet in analfalfa field at Fayoum Government. Insects, werereared on fresh alfalfaplants (*Medicagosativa*L.) at laboratory conditions of25 ± 2 C° ,65± 5% R.H.and 2nd and 4thinstars of the weevils larvae were selected for experiments.

Tested compounds

Commercial formulations of the following insecticides tested against 2nd and 4th instar larvae of*S.littoralis and H. brunneipennis* were obtained from the Agricultural Research Centre, Cairo, Egypt.
DipelDf (WP)6.4% : commercial product formulation contains 32X10^3 IU/ mg of *Bacillus thuringiensisvar.kurstaki*;W-Bus(WP) 8% : commercial product formulation contains 8X10^3 IU/ mg of *Bacillus thuringiensisvar.kurstaki* , Protecto (WP)9.4%: commercial product formulation contains 32X10^6 IU/ mg of *Bacillus thuringiensisvar.kurstaki*andBiofly (WP) : commercial product formulation contains 30X10^6 spores/ mg of *Beauverabassiana*.

Bioassay

The insecticidal activities of the tested Bt-formulations and fungi, each at four concentrations were prepared in distilled water and tested against 2nd and 4th instar larvae of *S.littoralis and H. brunneipennis* larvae using the dipping leaf technique **(Ahmed, 2009).** The leaves were first washed with distilled water and dipped in solution of the desired concentration of Btor fungi commercial formulations (DipelDf, W-Bus, Protecto and Biofly). Each leaf was dipped for 30 seconds, then placed individually in Petri- dishes (9 cm diameter) containing moistened filter papers to avoid desiccation of leaves. other castor bean leaves for treatment of*S. littoralis* and alfalfa for *H. brunneipennus* were treated with sterile distilled water for control. Then, ten larvae from each 2nd/ or 4th instars larvae were separately placed in each Petri dish for each treatment. Four Petri- dishes were used as replicates for each treatment and control. Larvae were allowed to feed for 48h. on treated leaves. Then these leaves were removed and replaced by another untreated ones.All Petri -dishes were kept at the above mentioned conditions. Larvae were examined daily for 7 days after treatment to determine the mortality percentages. Accumlative larval mortality was recorded and corrected using Abbott's formula (1925). Afterwards, the corresponding concentration probit lines were estimated in addition to determining 50% mortalities and slope values of tested compounds were also estimated. Data were analyzed by ANOVA and the means were separated using the Duncan's multiple range test **(Duncan, 1955).**
Fourty newly hatched 2nd, 4th instar larvae of each *S.lttoralis* and*H. brunneipennis* were fed as previously described (ten larvae/ four replicates) on leaves treated with the calculated LC_{50}starting of exposure was 2 days after applicationfor each of these compounds. The initial (2 days after application) and residual effect of Bt and fungi formulations at (4 ,6 and 8 days) after application against larvae were recorded at the end of the experiment (6) days.
The surviving larvae were transferred to other clean Petri- dishes, and supplied with untreated fresh castor bean leaves until pupation. Pupation and adult emergence percentagesafter treatment by the LC_{50}, and control were also determined.

Results and Discussion

Toxic effect of Bt andfungal formulations against 2nd and 4th instar larvae of *S.littoralis* and *H. brunneipennis*

Efficacies of the four concentrations of all tested insecticides on 2nd and 4th instar larvae of *S.littoralis* and*H. brunneipennis* at 7 day after treatment are presented in **table 1**. DipelDf, W-Bus and Protecto caused 100% mortality after treatment by highest concentration on the 2nd instar larvae of *H. brunneipennis*while treatment of 4th instar larvae caused 100, 90 and85%, respectively and 95, 85%mortality at Biofly. While the larval mortality was in the range 40 to 100 and 32.5 to 92.5 % on 2nd and 4th instar larvae of *S.littoralis*,respectively at Bt formulations and 77.5 , 60 % at Biofly. There were significant differences between the tested insecticides of both insects (F=75.08 ;df=3 for*S.littoralis*) and (F= 21.31 ; df= 3 for *H. brunneipennus*), respectively at 7 days post treatment. Also, there were significant differences betweenconcentrations andalso significant between 2nd and 4th instar larvae of both insect species (F=38.18 , ; df=3 for*S.littoralis*) and (F= 30.2 , ; df=3 for *H. brunneipennis*), respectively .

Table 1. Accumulated corrected mortality percentages after 7 days of treatment by some commercial Bt and fungi formulations against *S. littoralis* and *H. brunneipennis* 2nd and 4th instar larvae.

Formulations	Conc.gm/L	*Spodopteralittoralis*		*Hyperabrunneipennis*	
		Mortality %			
		2nd	4th	2nd	4th
Dipel DF	0.5	55def	45cde	90ab	75cd
	1	62.5bcde	60bc	92.5a	82.5bc
	2	87.5ab	85a	100a	100a
	4	100a	92.5a	100a	100a
Mean		76.25a	70.63a	95.63a	89.38a
W-Bus	0.5	52.5defg	40cde	70bc	60efg
	1	57.5cdef	50cd	90ab	75cd
	2	60cdef	55bc	100a	82.5bc
	4	82.5abc	77.5ab	100a	90ab
Mean		63.13b	55.63b	90ab	76.88b
Protecto	0.5	15g	12.5g	60c	50gh
	1	22.5fg	17.5fg	65c	52.5fgh
	2	27.5g	25efg	70bc	65def
	4	40efg	32.5defg	100a	85bc
Mean		26.25c	21.88d	73.75c	63.13c
Biofly	1	40efg	30defg	70bc	45h
	2	50efg	35def	80abc	57.5efgh
	4	55def	42.5cde	90ab	67.5de
	8	77.5abcd	60bc	95a	85bc
Mean		55.63b	41.88c	83.75bc	63.75c
F between treat. concen. and ages		1.37	1.52	1.28	1.06
Df	15				
F between concentrations	38.18			30.2	
Df	3			3	
F between treatment	75.08			21.31	
Df	3			3	

Data presented in table 1 indicate that the mortality percentage after treatment of the 2nd and 4th instar larvae of *S.littoralis* and *H. brunneipennis* increased gradually with increasing concentrations of all the insecticides.

The present results revealed that the tested Bt and fungus formulations had insecticidal activity against 2nd and 4th instar larvae of *S.littoralis* and *H. brunneipennis* larvae, where DipelDf highly killed the insect larvae both insect species , followed by W-Bus, Biofly and Protecto, respectively. These results agree with **Haggag, (2013)** who reported that DipelDf, Dipel 2x and Delfin highly killed *S.littoralis* larvae , followed by Agry, Protecto and Agerin, respectively . **Kaur (2000),** also, reported that *B.thuringiensis* applied for controlling of lepidopteran, dipteran and coleopteran insects for decades**. HerrnstadtandSoares (1989)** reported that *B.thuringiensis* 7.6x10^7 spores/ml solution , caused 80% mortality against alfalfa weevil. The surviving weevil larvae were stunted and ceased feeding. Lower concentrations resulted in minimal levels of mortality, but caused significant levels of feeding inhibition, these inhibited larvae will not survive to adulthood in the field. *B.thuringiensis* produced more than 93% mortality on first instar larvae of *Spodopterafrugiperda* and *Peridromasaucia* **(Alvarez*et al.*,2009)** .*B.thuringensis* Berliner is a promising agent for microbial control of agriculturally and medically important insects **(Souza*et al.*,2009).** The difference in activity might be due to the presence or absence of biologically active Cry toxins, their relative amounts and additive/ synergistic effect of these toxins in the formulations.**Shelton *et al.*, (1993).Karthikeyan**

andSelvanarayanan (2011) reported that the bioassay with *B. bassiana*against*S. litura,* percent mortality increased from 33.33 to 86.67 as the dose was increased from 0.15 to 0.25 %.

Susceptibility test

Table (2) reveals the LC_{50} values of the tested compounds against 2nd and 4th instar larvae of *S.littoralis* and *H. brunneipennis* recording 1.13&1.47; 2.75&6.47; 9.08& 14.90 and 2.015,5.05 gm/l, for DipelDf, W-Bus, Protecto and Bioflyagainst2nd and 4thinstar larvae of *S.littoralis* , respectively while those were 0.84, 0.14; 0.59, 0.44; 1.94& 1.41 and 2.53& 5.20 for *H. brunneipennis*, respectively.

According to the LC_{50}values,DipelDfwas the highest toxic to *S.littoralis* and *H. brunneipennis* than the other 3 compounds. The toxicity valuesof DipelDfwas significantly higher than that others.

Effects of LC_{50} of Bt and *B.bassiana*formulations on pupation and adult emergence percentage.

The initial and residual effects of Btand *B.bassiana* formulations at four time intervals (2, 4, 6, and 8 days) post application against 2nd and 4th instar larvae of *S.littoralis* and *H. brunneipennus*(at 6 days after treatment) are shown in **tables (3 and 4)** . Data in **table (3)** revealed that, treatment with all the tested compounds reduced pupation and adults emergence percentages and ,also, reduced the population of*S.littoralis* larvae compared to the control at initial and residual time intervals (2, 4,6 and 8 days) to record 51, 43.6 , 35.9 and 38.5 at 2 time; 37.5 , 30 , 25 and 27.5 at 4 days; 30, 27.5,15 and 20 at 6 days and 25.6, 20.5,7.7 and 12.8 % larval mortalities at the 8 days, respectively on 2nd instar larvae andrecored 42.5 , 37.5 , 30and 32.5 ; 30, 25, 20 and 20; 20, 17.5, 7.5 and 12.5; 17.5, 15, 5 and 10 at(2, 4, 6, and 8 days), respectively on 4th instar larvae.

The results in **Table (3)** indicated also that DipelDf and W-Bus decreased both pupation and adult emergence percentages at (2, 4, 6, and 8 days) more than Biofly and Protectocomparedto the control which recorded pupation and emergence rates of 97.5 and 100%, respectively.

The results of reduction percentage of *H. brunneipennis*population, pupation and adult emergence percentages after four indicating time intervals are summarized in **Table (4).** Data showed that the mean percentage ofcumulative larval mortality, pupation and adult emergence percentages of *H. brunneipennis*after four indicating time intervals of application(2, 4, 6, and 8 days) varied among the all treatments and control. The reduction was 60 , 55, 40, and 45; 45, 37.5, 20 and 25% larval mortality on the 2nd instar larvae and 52.5, 47.5, 35 and 47.5; 27.5, 20, 10 and 15 % larval mortality on the 4th instar larvae at theinitial time interval (2 day) and 6 days of application for DipelDf, W-Bus,Protecto andBiofly, respectively. These results agree with **El-Ghar*et al.*,(1995)** working with *Bacillus thuringiensis*andAbamectin against *S.littoralis* , with a pronounced decrease of pupation (36%) after Abamectin treatment. **Mohamed and Mahmoud, (2008)** reported that the rates of pupation and the emergence of mothsof *S.littoralis*were reduced by all tested insecticides (Dipel 2x, Agrin, BioGuard, Biofly and Spinosad), respectively as compared to the control. *Beauveriabassiana*caused significant decrease in pupal survival with the malformation among*S.littoralis* pupae (**Emara and Hefnawy,2000).** Hyphomycete fungi cause fatal infection to the immature stages of*S.littoralis*, this may due to the disruption of normal metabolism, and damage of target tissues such as fat body or alter hormone balance(**Meshrif*et al.*, 2007).**

From the above results and based on the LC_{50} values,DipelDf, proved as the highest toxic to*S.littoralis* and *H. brunneipennis* than that of the other compounds, followed by ,W-Bus,Biofly and Protecto.

Table 2 : Lethal concentration of Bt and fungi formulations against *S. littoralis* and *H. brunnipennus* larvae

Formulation	*S. littoralis*			*H. brunnipennus*		
	gm/L (spores/ml)			gm/L (spores/ml)		
		2nd	4th		2nd	4th
Dipel DF	LC_{50}	1.13	1.47	LC_{50}	0.14	0.84
	Slope	3.07	2.34	slope	0.69	1.34
W-Bus	LC_{50}	2.015	5.05	LC_{50}	0.44	0.59
	Slope	1.03	0.85	slope	0.39	1.48
Protecto	LC_{50}	9.08	14.90	LC_{50}	1.41	1.94
	Slope	0.90	0.80	slope	1.12	1.24
Biofly	LC_{50}	2.75	6.47	LC_{50}	1.20	1.53
	Slope	1.23	1.01	slope	1.40	1.63

Table 3: Initial and residual effect of the tested Bt and fungual formulations against 2nd and 4th instar larvae of *S. littoralis* at 6 days after treatment

Formulations	Accumulative larvae mortality % after indicated time intervals(days)											
	Initial kill			Residual effect								
	Accumulative 2 day			Accumulative 4 day			Accumulative 6 days			Accumulative 8 days		
	%Corrected mortality	%Pupation	% Adults emergence	%Corrected mortality	%Pupation	% Adults emergence	%Corrected mortality	%Pupation	% Adults emergence	%Corrected mortality	%Pupation	% Adults emergence
Dipel DF												
2nd	51	47	58	37.5	62	64	30	70	68	25.6	72	76
4th	42.5	58	61	30	70	71	20	80	75	17.5	82	78
W-Bus												
2nd	43.6	55	63	30	70	68	27.5	72	72	20.5	78	77
4th	37.5	62	68	25	75	76	17.5	82	79	15	85	79
Protecto												
2nd	35.9	63	76	25	75	80	15	85	82	7.7	90	89
4th	30	70	86	20	80	84	7.5	92	89	5	95	92
Biofly												
2nd	38.5	60	71	27.5	73	76	20	80	84	12.8	85	88
4th	32.5	67	81	20	80	81	12.5	87	83	10	90	91
Control												
2nd	-	95	94.7	-	97	95	-	97.5	94.9	-	97.5	100
4th	-	97.5	97.4	-	92.5	94.5	-	97.5	100	-	97.5	100

Table 4: Initial and residual effect of the tested Bt and *B.bassiana*formulations against 2nd and 4th instar larvae of *H. brunneipennis* at 6 days after treatment

Formulations	Accumulative larvae mortality after indicating time intervals(days)											
	Initial kill			Residual effect								
	Accumulative zero day			Accumulative 2 day			Accumulative 4 days			Accumulative 6 days		
	%Corrected larval mortality	%Pupation	% Adults emergence	%Correctedlarval mortality	%Pupation	% Adults emergence	%Correctedlarval mortality	%Pupation	% Adults emergence	%Correctedlarval mortality	%Pupation	% Adults emergence
Dipel DF												
2nd	60	40	44	52.5	47	47	50	50	60	45	55	64
4th	52.5	47	53	45	55	59	32.5	67	63	27.5	72	69
W-Bus												
2nd	55	45	50	47.5	52	52	45	55	55	37.5	62	68
4th	47.5	52	57	40	60	62	27.5	72	66	20	80	72
Protecto												
2nd	40	60	56	40	60	58	27.5	73	69	20	80	78
4th	35	65	65	27.5	72	69	20	80	75	10	90	81
Biofly												
2nd	45	55	54	40	60	58	32.5	67	63	25	75	73
4th	47.5	52	62	35	65	69	25	75	70	15	85	76
Control												

2nd	-	95	97	-	92.5	97	-	95	100	-	92.5	100
4th	-	95	100	-	97.5	92	-	97.5	100	-	97.5	100

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الملخص العربى

تقييم لبعض المستحضرات التجارية على دودة ورق القطن وسوسة ورق البرسيم

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تم تقيم فاعلية بعض المستحضرات البكتيرية (الدايبل دى إف ، دابيلو بص، بروتيكتو) والفطرية بيوفلاى ضد كل من العمر اليرقى الثانى والرابع لدودة ورق القطن و سوسة ورق البرسيم تحت الظروف المعملية. وأوضحت النتائج ان نسب الموت عند استخدام المستحضرات البكتيرية على كل من العمر اليرقى الثانى والرابع لدودة ورق القطن تراوحت بين ٤٠ – ١٠٠ % ، ٣٢.٥ – ٩٢.٥ فى حين وصلت الى ١٠٠ ، ٨٥ و١٠٠ % على العمر اليرقى الثانى والرابع لسوسة ورق البرسيم على التوالى عنداستخدام أعلى تركيز بعد ٧ ايام من المعاملة مقارنة ب ٧٧.٥ و ٦٠ % على دودة ورق القطنو ٩٥ ، ٨٥ % لسوسة ورق البرسيم عند استخدام البيوفلاى.

وبناء على قيم كان الدايبل افضل المستحضرات المستخدمة ضد كل مندودة ورق القطن و سوسة ورق البرسيم مقارنة بالمستحضرات الاخرى.

كما إنخفضت نسب خروج كل من العذارى والحشرات الكاملة عند المعاملة بالمستحضرات السابقة مقارنة بالكنترول.