

Field and Biochemical Studies of Bio- and Chemical Pesticides on the Cotton Leafworm, *Spodoptera littoralis* (Boisduval) on Sugar Beet

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ABSTRACT

Cotton leafworm, Spodoptera littoralis (Boisd.) is a serious lepidopterous pest affecting several economic plant families leading to lowering in crop yields as well as crop quality through its larval stages Efficacy of Dipel 2x®as bio-insecticide and Hamer® and Jasper® as biorational insecticides were investigated against S. littoralis larvae under field conditions in Egypt throughout two seasons August 2018 and 2019 on Sugar Beet. Results showed that the population of S. littoralis larvae during both seasons reduced after insecticide application. The highest reduction in infestation percentages was recorded with Jasper® followed by Hamer[®] then Dipel 2x[®]. It recorded 96.30%, 90.06%, 80.32%, 78.70% and 68.52%, 67.31% with Jasper, Hamer and Dipel 2x[®] during 2018 and 2019, respectively. The overall reduction in infestation percentages was 87.78%, 86.46%, and 64.76%, 60.71 % and 53.67%, 49/.10% for Jasper, Hamer, and Dipel 2x[®] during 2018 and 2019 Sugar Beet growing seasons, respectively. The activity of acetylcholinesterase (AChE) and Glutathione S- transferase (GST) enzymes play an important role in insect immune response compared to control against susceptibility of field strain 3rd instar larvae of S. littoralis was determined. Our results revealed that Dipel 2x[®] was the most potent bioinsecticide in both crop yields and quality due to its larval activity followed by Hamer[®] then Jasper[®] among the selected insecticides. The present work discusses the role of biorational and biological insecticides application in integrated pest management and biochemical studies were carried out on 3rd instar larvae of S. littoralis (Field strain) to determine AChE and GST enzyme activity.

INTRODUCTION

Sugar beet, *Beta vulgarias* L.(Chenopodiaceae) is considered as the second most important source of sugar production In Egypt after Sugar cane. Kafr El-Sheikh Governorate represents the utmost producing source of Sugar beet; including more than 50% of the sugar beet -cultivated area (Abou El-Kassem, 2010). Crop importance and uses relies on the high sugar content of root (Rashid, 1999), making it a commercial source of sugar as well as ethanol production in temperate countries (BSRI, 2005). Egyptian Cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera :Noctuidae) is

a major (quarantine) pest affecting economic important plant families leading to lowering in crop yields as well as crop quality (OEPP/EPPO 1981). Among the crops infested by this insect is the Sugar beet, Beta vulgaris L. (Chenopodiaceae). During the growing season that starts from mid -August, the average recorded temperature one month later (during September) assisted in the infestation of Sugar beet seedlings by S. littoralis larvae causing great losses and resulting in forming bare areas in the field in case of heavy infestation (El-Mahalawy, 2011). The infestation rate is considered critical when reaching 20%. Concerning pest management on edible crops, it is of major concern to consider both pest killing efficiency, environmental contamination, wildlife, and public health concerns, as well as food safety (El-Geddawy et al. 2014). Moreover, the indiscriminate use of insecticides results in increasing insecticide resistance, hence the need for biologically safe insecticide affording environmental sustainability is a must. Integrated pest management includes applying both biological and chemical control measures with special focusing on those non- conventional (bio-rational) insecticides (Pedigo 1996). The best candidate tactic for lowering resistance is using biocides and insecticides with novel modes of action. The entomopathogenic bacteria B. thuringiensis ,var kurstaki, has been widely used efficiently against laboratory and field populations of lepidopterous pests (Obeng-Ofori and Sackey, 2003; Said et al., 2012; Abd El-Kareem and Ibrahim, 2015; Fetoh, et al. 2015; Hamama et al., 2015; Darabian and Yarahmadi, 2017, Yasmin, El Fargany, 2019). The field efficacy of emamectin benzoate and thiacloprid was evaluated against many lepidopteran pests as Cydia pomonella (Brunner et al., 2005), Pieris rapae (Muthukumar et al., 2007 and Said et al., 2012). S. litura (Tong et al., 2013) S. littoralis (Fetoh et al., 2015).

Acetylcholiesteras (AChE) enzyme is a key enzyme in the insect nervous system that hydrolyzes acetylcholine neurotransmitter to terminate nerve impulses. (AChE) known to play a major part in insecticide resistance (Zhu *et al.*, 2000, Russell *et al.* 2004). Glutathione *S*- transferase (GST) enzymes have attracted attention in insects because of their involvement in the defense towards insecticides (Clark *et al.* 1986; Grant &Matsumura 1989; Reidy *et al.* 1990; Fournier *et al.* 1992). Rumpf *et al.* (1997) observed that there is a correlation between the degree of acetylcholinesterase AChE and GST inhibition and corresponding mortality caused by insecticide in lacewings.

The present work discusses the role of biorational and biological insecticides application in integrated pest management and biochemical studies were carried out on 3rd instar larvae of *spodoptera littoralis* (field strain), to determine AChE and GST enzyme activity.

MATERIALS AND METHODS

The suppressive effect of Hamer[®] (Thiacloprid), Jasper[®] (Emamectin benzoate), and Dipel $2x^{\$}$ (*B. thuringeinsis*) commercial insecticide against *S.littoralis* larval population were evaluated in sugar beet field with early infestation during 2018 and 2019.

Insecticides:

1- Dipel $2x^{(0)}$, *B. thuringeinsis* var. *kurstaki*, (32, 258 Potency I.U. / mg) WP, rate= 200 g/feddan, obtained from the Bio-insecticide Production Unit, Plant Protection Research Institute, Agriculture Research Centre, Giza, Egypt.

2- Hamer[®], Thiacloprid 480 g/l S.C., Chloro-nicotinoid. 480 g/l S.C., China.

3- Jasper[®], Emamectin benzoate, Avermectin, 480 g/l S.C., China.

Fied Application:

The field experiments were constructed in sugar beet fields infested with S.

littoralis at Talaat-El Agamy experimental farm, henno, Kafr El-Sheikh, Egypt. Studies covered two successive growing seasons during 2018 and 2019. 1/100 feddan was used for experimental application applying a complete randomized block design. Each treatment was applied to an area of about 42 m^2 , four plots per treatment as well as untreated (control) area sprayed with water only. Separating vegetation between plots of 2 rows of sugar beet plants were left untreated. Application insecticides were applied at sunset using knapsack motor sprayer (20L capacity). 10 plants/plot/treatment were examined before insecticide application and after 2, 3, 5 days after bacterial insecticide treatments. The numbers of *S. littoralis* larvae found were recorded and the percentage of infestation reduction was calculated according to Henderson and Tilton's formula. (Henderson and Tilton, 1955) as follows:

Reduction %= $\left\{1 - \frac{n \text{ in Co before treatment } x n \text{ in T after treatment}}{n \text{ in Co after treatment } x n \text{ in T before treatment}}\right\}$ ×100. N: Insect population, T: treated, Co: control

Biochemical Analysis:

Biochemical studies were done to examine the (AchE) acetylcholinesterase and Glutathione S-Transferase (GST) enzymes of treated insects.

1-Field Strain:

Field strain of 3^{rd} instar larvae of *S. littoralis* was collected from Syngenta localities in Qulaubyia Governorate, Egypt, and treated with quarter recommended concentration of each insecticide for further biochemical analysis. Leaf of castor bean was dipped in biorational insecticides; Hamer[®], Jasper[®], and bio-insecticide Dipel $2x^{®}$ for 10 sec. and left to dry under laboratory conditions. Leaves were put on the bottom of plastic cans covered with a sieved lid, then 30 larvae released in 3 replicates for each pesticide, while control leaves dipped in distilled water only. The live larvae were collected after 24 h for pesticide and 48 h for biopesticide in open dwarf at -20° c for use in biochemical analysis.

2-Sample Preparation of Insects for Analysis: -

The insects were prepared as described by Amin (1998). Sample insects were homogenized in distilled water (50 mg /1 ml), then centrifuged at 8000 r.p.m. for 15 min at 2 °C in a refrigerated centrifuge. The deposits were discarded and the supernatants, which are referred to as enzyme extract, stored at least one week without appreciable loss of activity when stored at 5^{0} C.

3-Enzyme Assay:

a-AchE (acetylcholinesterase) activity was measured according to the method described by Simpson *et.al.* (1964).

b-Glutathione S-Transferase (GST) catalyzes the conjugation of reduced glutathione (GSH) with 1-chloro 2,4-dinitrobenzene (CDNB) via the -SH group of glutathione. The conjugate, S-(2,4-dinitro-phenyl)-L-glutathione could be detected as described by the method of Habig *et al.*(I974).

4-Statistical Analysis:

Data were presented as the mean number of collected larvae per treatment and percent reduction in the infestation. Statistical analysis was followed by the analysis of variance (ANOVA) at p=0.05 using Minitab v. 16 software and the results of biochemical analysis were pooled from triplicate determinations by using costat statistical software (cohort software, Berkeley). When the ANOVA statistics were significant (P <0.01), the means were compared by Duncan's multiple range test.

RESULTS AND DISCUSSION

Field Studies:

Effect of Tested Insecticides on S. littoralis Larval Population in 2018:

The suppressive effects of Hamer[®], Jasper[®], and Dipel $2x^{\$}$ against the larval population of *S. littoralis* were evaluated in the sugar beet field with early infestation during 2018 and 2019. Under normal, (control plot), the larval population increased gradually during the first 4 days then increased after 5 days during the two studied growing seasons (Tables 1 & 2). Treatment with bacterial insecticide, Dipel $2x^{\$}$, the application showed a significant reduction in the mean numbers of larvae compared to the control after 48h post-treatment reached to 40.84%, then increased to 68.52% after 5 days post-treatment. Both chemical insecticides, Jasper[®] and Hammer[®], significantly reduced larval population 24hr post-treatment, in comparison with larval counts before treatment. The suppressive effect extended to 5 days post-treatment. The reduction in 1 day, while Hamer[®] was slower in its effect causing only 46.88%, the reduction in the larval population increased to 80.32% in case of Hamer[®] and 96.30% in case of Jasper[®] after 5 days post-treatment (Table 1).

Tables 1: Mean reduction of *S.littoralis* larvae population in treated and untreated plots of Sugar beet field in 2018. 1st Season

	Untreated Area	Days after treatments							
Treatment		1 DAY		2 DAYS		3 DAYS		5 DAYS	
	Mean ±S.D.	Mean ±S.D.	Reduction %	Mean ±S.D.	Reduction %	Mean ±S.D.	Reduction %	Mean ±S.D.	Reduction%
Dipel 2x®	13.00± 2.16 ª			9.50± 1.91 ab	40.84	8.75± 2.21 b	51.65	6.50± 1.29 ^b	68.52
Hammer®	12.00 ± 1.63^{a}	7.00 ± 1.82^{b}	46.88			5.50± 1.29 b	67.08	3.75 ± 1.70^{b}	80.32
Jasper®	12.50 ± 2.08 $^{\rm a}$	2.75 ± 1.25 ^b	79.96			2.25 ±0.95 b	87.07	0.75± 0.50 ^b	96.30
Control	12.75± 1.70 ^b	14.00± 1.41 ^b		15.75 ± 3.77^{ab}		17.75±3.09 ab		20.25 ± 2.75^{a}	

Means in the same raw followed by different letters are significantly different, P≤0.05

Effect of Tested Insecticides on S. littoralis Larval Population in 2019:

The same pattern was observed in the second season (2019) with a lower magnitude of the reduction. Results presented in table (2) showed also that Jasper[®] 24h post-treatment caused the highest larval mortality compared with the control. The reduction in larval population showed a fast-promising effect of Jasper[®] recording 90.06 % reduction in 5 days, while Hamer[®] was slower in its effect causing only 78.70% after the same period. Also, it is noticed that the reduction in larval numbers caused by Dipel $2x^{\$}$ started to increase after 48h post-treatment reached 27.14% and 67.31% after the 5th-day post-treatment.

Tables 2: Mean reduction of <i>S.littoralis</i> larvae population in treated and untreated plots
of Sugar beet field in 2019. 2 nd Season

	Untreated Area	Days after treatments							
Treatment	Mean ±S.D.	1 DAY		2 DAYS		3 DAYS		5 DAYS	
		Mean ±S.D.	Reduction %	Mean ±S.D.	Reduction %	Mean ±S.D.	Reduction %	Mean ±S.D.	Reduction %
Dipel 2x®	10.75 ± 2.21^{a}			$9.50 \pm 2.64^{\mathrm{ab}}$	27.14	$7.25 \pm 2.63^{\mathrm{ab}}$	50.76	$5.50{\pm}~2.08^{\rm b}$	67.31
Hammer®	10.50±1.29 ª	7.75± 2.5 ^{ab}	34.71			$4.5\pm2.64~^{b}$	68.71	$3.50\pm1.29^{\text{b}}$	78.70
Jasper®	11.25± 2.21ª	2.25± 0.95 ^b	82.31			2.00 ± 0.81^{b}	87.02	1.75± 1.95 b	90.06
Control	11.50± 1.91 ^b	$13.00{\pm}\;0.81^{ab}$		14.00± 3.65 ab		$15.75{\pm}4.34^{ab}$		18.00 ± 2.16^{a}	

Means in the same raw followed by different letters are significantly different, P≤0.05

The overall reduction in infestation was 87.78%, 86.46% with Jasper®, 64.76%,

60.71% with Hamer[®], and 53.67%, 49.10% with Dipel 2x[®], during 2018 and 2019 seasons respectively. (Table 3). The field effect of bacterial insecticides on lepidopteran pests as S. littoralis and Sesamia cretica population reduction proved its efficiency using commercial formulations like Xentari[®], Dipel 2x[®], Agerin[®] and protecto[®] (El –Zoghbey et al., 2003; Radwan et al., 2004; Salem, 2011; Said et al., 2012 and Abd El-Kareem and Ibrahim, 2015). Also, our results agree with Jat, et al. (2017) who found that B. thuringiensis caused a reduction of larval population by 56.09 and 55.24% during 2013,2014 on cabbage crops. During the investigation, the data revealed better efficacy of emamectin benzoate and this agrees with Nukala, et al. (2015) who investigated bioefficacy of nine modern insecticides under field conditions against S.litura and revealed that emamectin benzoate, chlorpyriphos, Cypermethrin, and Chlorantraniliprole were found most effective. (Muhammad, et al., 2018). Emamectin benzoate was proved to be safer for parasitoid, which caused 18% mortality after 48 hrs exposure during laboratory assessment. Emamectin benzoate is the salt of emamectin effects on the nervous system of arthropods by increasing chloride ion flux at the neuromuscular junction, resulting in irreversible paralysis. Also, it affects the glutamate-gated chloride channel agonist (Dunbar et al., 1998). It is observed that Emamectin benzoate is a safer insecticide for Trichogramma. Looking at the efficacy of all the insecticides emamectin benzoate, Thiacloprid, and the Bacillus thuringiensis kurstaki can be suggested to the farmers for the management of S. littoralis sugar beet. The present and previous studies support the benefit of using bacterial insecticides as a part of integrated pest management (IPM) for being an effective and environmentally safe control agent.

Tables 3: Overall reduction	of S. <i>littoralis</i> la	arvae in seasons	2018 and 2019	in sugar beet
field.				

Treatment	Overall reduction In Season 2018%	Overall reduction In Season 2019%		
Dipel 2x®	53.67%	49.13%		
Hammer®	64.76%	60.71%		
Jasper®	87.78%	86.46"%		

Biochemical Studies:

Effect of Treating 3rd Instar Larvae of S. littoralis (field strain) With Bioinsecticide and Insecticisdes On Glutathione S-transferase and Actylcoliesterase Studied: a-Acetylcholinesterase (AChE):

Treatment of the 3rd instar larvae (field strain) with Hamer[®], Jasper[®], and Dipel 2x[®] resulted in a significant increase in Acetylcholinesterase (AChE) enzyme activity with Dipel $2x^{\otimes}$ in comparison to control. Dipel $2x^{\otimes}$ treated larvae showed significantly higher enzyme activity but decreased in the case of Jasper[®] followed by Hamer[®] treatment after 24 h post-treatment in comparison to control (Table 4).

b- Glutathione S-transferase (GST) Activity:

Bioinsecticides Dipel 2x[®] treated larvae significantly increased the GST enzyme activity after 48 h post-treatment in comparison to control. But treatment with insecticides higher enzyme activity in Hamer[®] followed by Jasper[®] in comparison to control (Table 4).

Obtained results showed a significant increase in the enzyme activity of (AChE) after 48 h exposure to Dipel 2x[®] 421 µg ACh Br/min/ml compared with control 400 µg Ach Br/min/ml. But in case of both insecticides (Jasper[®] and Hamer[®]) decreased in AChE enzyme activity was observed 310.6 and 357 μ g ACh Br/min/ml), respectively compared with control 394.5 μ g ACh Br/min/ml., after 24 h exposure. The obtained results are in agreement with those found by Abo Elghar (2005) who stated that AChE enzyme activity decreased in field strains than that of the susceptible strain against cotton leafworm. Generally, AChE from the field strain exhibited a higher insensitivity to the organophosphorus insecticides than the carbamate insecticides. The present study disagreement with those found by (Nour El-Hoda, *et, al.,2012*) showed significantly higher AChE enzyme activity in chlorpyrifos and profenophos-treated field strain than the (Lab-susceptible) strain.

Tested Compounds	AChEactivity (µg Ach Br/min/ml) (Mean ±S.D)	GST activity (µmole/min/ml) (Mean ±S.D)
Dipel 2x®	421±14.7ª	77.6 ± 6.2^{b}
Control*	400.66 ±12.35ª	$50.6\pm4.25^{\text{c}}$
Jasper®	$310.6 \pm 3.5^{\circ}$	70.6 ± 4.1^{b}
Hammer®	357 ± 9.3^{b}	111.6 ±1.2ª
Control**	$394.6\pm7.4^{\mathtt{a}}$	$69.6\pm3.2^{\mathrm{b}}$
F values	25.39***	49.73***
L.S.D.	29.0399	11.780

Table 4: AChE and GST enzyme activity in 3 rd instar larvae of <i>S. littoralis</i> (Field strain)
using bioinsecticids and insecticides compounds

-Means of the same column followed by different letters are significantly different, P \leq 0.05. *after 48 hours ** after 24 hours

Glutathione S-transferase (GST) is a group of soluble detoxification enzymes that catalyze the conjugation of reduced glutathione with various compounds containing an electrophilic center, including insecticides (Wilce and Parker, 1994). The present study showed a significant increase in the enzyme activity of (GST) after 48 h exposure to Dipel 2x[®] 77.6 µmole/min/ml compared with control of 50.6 µmole/min/ml. Insecticides (Jasper[®] and Hamer[®]) caused a significant increase in the GST enzyme activity after 24h post-treatment 70.6 and 111.6 µmole/min/ml., respectively compared with control 69.6 µmole/min/ml. The obtained results are agreement with those found by (Nour El-Hoda, et al., 2012). Higher activity of glutathione-S-transferase (GST) has been observed in chlorpyrifos and profenophos- treated field strains, over that of the lab-strain of PBW larvae. The obtained results are in disagreement with those found by Wang et al. (2009) found that GST might be not important in conferring spinosad resistance in the S. exigua field population. Results are disagreement with Hamama and Fergani (2019) who recorded a decrease in GST enzyme activity after treatment of 3rd instar larvae laboratory to thiacloprid, indoxacarb, and Bacillus thuringiensis, Emamectin benzoate did not affect GST enzyme activity.

CONCLUSION

The outcome of these studies may help in proper insecticide selection for better insecticide resistance management.

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ARABIC SUMMARY

الدراسات الحقلية والبيوكيميائية للمبيدات الحيوية والكيميائية على دودة ورق القطن على بنجر السكر

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تعتبر دودة ورق القطن من آهم آلأفآت الحلقية الخطيرة آلتي تؤثر على العديد من النباتات الاقتصادية وقد تم استخدام دايبل تواكس كمبيد حشري حيوي والهامر والجاسبر كمبيدات كيميائية لمكافحة هذة الافة خلال الموسمين الموسمين انخفض بعد استخدام المبيدات الكيميائية. حيث تم تسجيل أعلى نسبة انخفاض في الإصابة مع جاسبر يليه هامر ثم دايبل تواكس وسجلت ٩٦,٣٠ ٪، ٢٩,٠٠ ٪، ٢٢، ٨ ٪، ١٨,٧٠ ٪ و ٢٥,٨٥ ٪، ٢٧,٣١ ٪، مع جاسبر ، هامر و دايبل تواكس وسجلت ٩٦,٣٠ ٪، ٢٠,٠٩ ٪، ٢٢،٣٠ ٪، ١٨,٧٠ ٪ و ٢٥,٨٥ ٪، ٢٧,٣١ ٪، مع جاسبر ، هامر و دايبل تواكس وسجلت ٢٠١٨ رو ٢٠١٩ رو ٢٠١٩ ، على التوالي. كان الانخفاض الكلي في نسب الإصابة مواسم زراعة بنجر المكر والميدات الكيميائية. حيث تم تسجيل أعلى نسبة انخفاض في الإصابة مع جاسبر مواسر ثم دايبل تواكس وسجلت ٢٠,٣٥ ٪، ٢٠,٠٦ رو ٢٠،٩٠ ٪، ٢٢،٧٩ ٪ و ٢٥,٨٥ ٪ مع جاسبر يليه مواسم زراعة بنجر السكر ٢٠١٨ و ٢٠,٧٦ ٪ و ٢٠,٧٦ ٪، على التوالي. كان الانخفاض الكلي في نسب الإصابة مواسم زراعة بنجر السكر ٢٠١٨ و ٢٠,١٠ ، على التوالي. كان الانخفاض الكلي في نسب الإصابة مواسم زراعة بنجر السكر ماما و ٢٠١٠ معلى التوالي. كان لنشاط إنزيم الأسيتيل كولينستراز وأنزيم الجلوتاثيون دورًا مهمًا في الاستجابة المناعية عند معاملة السلالة الحقلية ليرقات العمر الثالث لحشرة دودة ورق القطن، حيث كشفت النتائج ان دايبل تواكس كان المبيد الحيوي الأكثر تاثيرا علي الحشرة يلية مركب الهامر ثم الجاسبر بين المبيدات الحشرية محل الدرسة.