Comparison of Efficiency of Some Compounds and their Biochemical Effects against Cotton Leaf Worm, *Spodoptera littoralis* (Boisd.) in the Field By

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ABSTRACT

The cotton leaf worm Spodoptera littorals (Boisd.) is one of the most notorious and destructive pests in Egypt. The aim of this work estimating the effectiveness of different types of compounds, and comparison between them and test their sensitivity, by estimating the percentage of reduction of larvae after spraying in the field out at different intervals and compare its efficiency against cotton leaf worm in each period, as well as follow up the biochemical changes of larvae. The present work was conducted during two successive seasons 2012 and 2013 in the area of Nubaria on the sugar beet crop by spraying Challenger (Chlorfenapyr), Avant (Indoxacarb) and three of insect growth regulators(Dimlin (Diflubenzuron), Match and Saimex (Lufenuron)), and estimated the percentage of reduction of larvae of cotton leaf worm after different intervals which after 24 hours to estimate the immediate effect, as well as after 3.5and 7 days to evaluate the latent effect. In the biochemical studies evaluate the total protein and enzyme activity of chitinase for larvae during the two seasons. The results showed that the Challenger given immediately highest effectiveness after 24 hours while the rest of the compounds were less and slower effect and then began to show its effectiveness in percentage of reduction after 3.5 and 7 days. The results showed during the two seasons, the average percentage of reduction of larvae after treatment with Challenger was 96. 38% while with Dimlin, Match , Saimax and Avant were 66.53 - 64.87 - 63.11 - 70.38%, respectively, and also the biochemical study showed that the tested compounds caused varying decrease in the total content of protein in two seasons (2012 and 2013). The effect of compounds on the efficiency of chitinase activity showed that insect growth regulators increase in the activity, while the Changer and Avant caused decrease of chitinase activity compared to untreated larvae during two seasons

Key words: Spodoptera littoralis, Chitin synthesis inhibitors, Lufenuron, Chlorfenapyr, Indoxacarb and Diflubenzuron.

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INTRODUCTION

Insect pests are a major constraint on crop production, especially in developing countries. Sugar beet quality is of great economic importance. Several numbers of insects attack this crop caused considerable damage in its yield, the cotton leaf worm, *Spodoptera littoralis* (Boisd) is considered as the most serious and destructive phytophagous pest in field, vegetable and ornamental crops in Egypt. The continues and unwise use of conventional insecticides to control agriculture pests usually leads to adverse effects on non target organisms and the development of resistance in the target pests (Ishaaya and Horowitz, 1995).

The new approaches for controlling agricultural pests is the development of novel compounds affecting specific processes in insects, Challenger (Chlorfenapyr) is relatively new proinsecticides which are discovered as a natural insecticide. (Braham. et. al 2012).

Challenger is used commercially for termite control and crop protection against a variety of insect and mite pests (Sheppard and Joyce 1998). Challenger is a pro-insecticide and oxidative removal of the N-ethoxymethyl group of chlorfenapyr by mixed function oxidases leads to a toxic form identified as CL 303268 which functions to uncouple oxidative phosphorylation in the mitochondria, resulting in disruption of ATP production and loss of energy leading to cell dysfunction and subsequent death of the organism. This molecule has low mammalian toxicity and is classified as slightly hazardous insecticide as per WHO criterion (Guessan et al 2009) .Due to its novel mode of action, Challenger is unlikely to show any cross resistance to standard neurotoxic insecticides as observed in *Anopheles gambiae*, *Anopheles quadrimaculatus* (Pridgeon 2009), *Aedes aegypti* (Paul et al 2006) and *Culex quinquefasciatus* (Oxborough et al 2010).

Avant is a non-systemic, synthetic organophosphate replacement insecticide used to control sucking insects. Avant affects insects from direct exposure and through ingestion of treated foliage/fruit. Once Avant is absorbed or ingested, feeding cessation occur. It kills by binding to a site on sodium channels and blocking the flow of sodium ions into nerve cells. The result is impaired nerve function, feeding cessation, paralysis, and death (Brugger, 1997).

The insect growth regulators (IGRs), can be grouped according to their mode of action as: chitin synthesis inhibitors and substances that interfere with the action of insect hormone (Tunaz, H. and N. Uygun, 2004). Chitin synthesis inhibitors (CSIs) These compounds are effective suppress of development for the entire life cycle on insects (Gelbic et al 2011). Chitin synthesis inhibitors are compounds affecting specific processes in insects (Berry et.al. 1993). The insect growth regulator for controlling the major insect pests in crops. (Perveen, F. 2011). Dimilin interferes with chitin synthesis (Cohen1987). It also decreases food consumption and growth rate (Mulder and Gijswijt 1973, Ascher and Nemny 1976, Radwan et al. 1986). The lufenuron is insect growth regulator that interferes with chitin synthesis, disrupt hormonal balance with exchanging in molting process, and inhibit the insect's growth (Oberlander and Silhacek, 1998). Lufenuron treated larval instars were profoundly growth inhibited accordingly the weight gain was drastically reduced, it had also a latent effect on egg hatchability was strongly observed (Manal M.2012).

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MATERIAL AND METHODS

Test insects

A field strain (F-strain) of the cotton leaf worm *Spodoptera littoralis* (Boisd) (Lepidoptera: Noctuidae) were obtained from Nubaria region, Behira Governorate of larvae insects used in the present work.

Insecticides and insect growth regulators used

The following five insecticides were applied along with one control to study their effectiveness on these pest populations.

Challenger

Common name: Chlorfenapyr Trade name: Challenger (24 % SC) belongs to the pyrrole class is obtained from shoura Chemical Company. It is insecticide and acaricide .50cm3/100 liter water.

Structural formula



Chemical name: 4-bromo-2-(4-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-1Hpyrrole-3-carbonitrile.

Indoxacarb

Common name: Indoxacarb Trade name: Avant 15%EC

Chemical group of the Oxadiazin. Use of the Substance Insecticide 50 cm3/liter **Structural formula**



Chemical name: Polyethylene Oxide Mono. Tris (Alpha-Methylbenzyl) Phenyl. Ether Dimilin: Common name: Diflubenzuron Trade name: Dimilin48% EG.

It is a benzyl phenyl urea (BPU) chitin-synthesis inhibitor (CSI) and insect growth regulator (IGR) is formed by Ishihara Sangyo Kaisha, Japan. 125 cm3/fedan.

Structural formula



Chemical name: 1-(4-chlorophenyl)-3-(2, 6-difluorobenzoyl) urea

A. Match and b. Saimix

Common name: Lufenuron Trade name: Match (50%WG) 40gm/fedan and Saimix (5%EC) of the benzoylurea, the same active ingredient (Lufenuron) were obtained from Sumitomo Chemical Company 160 cm 3/ fedan.

Structural formula



Chemical name: 1-[2, 5-dichloro-4-(1, 1, 2, 3, 3, 3-hexafluoro-propoxy)-phenyl]-3-(2, 6-difluorobenzoyl)-urea

Field Experiments

The experiments were conducted at Nubaria region, Behira Governorate to evaluate the field efficiency of five insecticides against cotton leaf worm, *Spodoptera littoralis* (Boisd). The field area was cultivated with sugar beet plants (tomson) on seasons 2012 and 2013 the normal agricultural practices were applied. The experimental area was divided into plates of 1/16 feddan (262.5 m2). The treatment was arranged in randomized complete blocks design (RCBD) with four replicates each. A motor sprayer was used. The volume of spray solution was recommended doses/Fadden. The number of larvae were recorded on one meter lengthwise for five times (four at corners and the last one on plot center), before the spray and on 1,3,5 and 7 days after the spray.

The percentage of reduction in the population density of insects was estimated according to Henderson and Tilton (1955).

Biochemical studies

Sample Collection: Total body tissue samples were collected from late 6th instars treated as 4th instars fed for 24 hour on sugar beet leaves are sprayed with recommended dose values of five compounds. The samples were obtained by homogenizing the 6th instars larvae representing 1gm larval body weight in 5ml distilled water. The samples were collected in cold tubes (on ice) until analysis samples of non-treated also were prepared in the same manner.

a- Determination of Total protein: Total proteins were determined by the method of Bradford (1976).

b- Determination of chitinase activity: Colloidal chitin was prepared according to Bade and Stinson. (1981).

Statistical analysis

The statistical significance of differences between individuals means were determined by using one way ANOVA test. Levels of significance of each experiment was stated to be significant at (P = 0.05). In addition the control index was calculated according to Sun (1950).

RESULTS

Field studies

The data in table (1) indicated that the Immediate and latent affect of the five compounds against *Spodoptera littoralis* on the sugar beet crop for the season 2012 after different intervals under field conditions:

1- The reduction rates of the challenger after 24 hours recorded 96.24% while Dimilin, Match, Saimix and Avant recorded 8.5, 7.89, 6.38, and 10.49 respectively.

2- Whereas, reduction rates of the challenger after 3 days of spray recorded 95.93, while Dimilin, Match, Saimix and Avant 83.13, 81.78, 80.74, 90.53 respectively.

3- While after five days of spray; the infestation reduction rates recorded Challenger 97.58, Dimilin, Match, Saimix and Avant recorded 85.50, 83.41, 80.35 and 90% respectively.

4- Also the reduction rates after seven days of spray the challenger recorded 97.01, while Dimilin, Match, Saimix and Avant recorded 86.81, 84.68, 81.97 and 91.21 respectively.

The data in table (2) indicated that the Immediate and latent affect of the five compounds against *Spodoptera littoralis* on the sugar beet crop for the season 2013 after different intervals under field conditions.

1- The reduction rates of the challenger after 24 hours recorded 95.65 % while Dimilin, Match, Saimix and Avant recorded 8.76, 7.60, 6.22, 11.08respectively.

2- Whereas, reduction rates of the Challenger after 3 days of spray recorded 96.18, while Dimilin, Match, Saimix and Avant 85.25, 83.34, 81.79, 92.11 respectively.

3- While after five days of spray; the infestation reduction rates recorded Challenger 96.24, while Dimilin, Match, Saimix and Avant 86.67, 84.80, 83.50, 89.59 respectively.

4- Also the reduction rates after seven days of spray the challenger recorded 96.91, Dimilin, Match, Saimix and Avant 87.63 , 85.46, 83.95, and 88.04 respectively.

The data in table (3) indicated that the mean reduction for Challenger during two seasons (2012&2013) recorded 96.38, while Dimilin, Match, Saimix and Avant recorded 66.53, 64.87, 63.11 and 70.38 respectively.

Table 1. Immediate and latent effect of the five compounds against *Spodoptera littoralis* on the sugar beet crop for the season 2012 during different time intervals after treatment under field conditions.

Insecticides	The men reduction % in larval population at indicated days after					
	spray					
	24 Hours	3 days	5 days	7 days		
Challenger	96.24	95.93	97.58	97.01		
24%EC	±1.04	±0.46	±1.23	±1.03		
Dimilin 48%EG	8.58	83.13	85.50	86.81		
	±0.50	±1.30	±1.68	±1.51		
Match 50%WG	7.89	81.78	83.41	84.68		
	±0.49	±1.08	±1.06	±1.28		
Saimix 5%EC	6.38	80.74	80.35	81.97		
	±0.83	±0.52	±0.53	±0.31		
Avant 15%EC	10.49	90.53	90.00	91.21		
	±1.09	±1.33	±1.36	±1.34		
F. value	7551.63 *	471.144*	512.077 *	1894.20*		
L.S.D	1.310	0.6469	0.690	0.4046		

Insecticides	The men reduction % in larval population at indicated days after spray			
	24 Hours	3 days	5 days	7 days
Challenger	95.65 a	96.18 a	96.24 a	96.91a
	±1.68	±1.01	±1.23	±1.34
Dimilin	8.76 c	85.25 c	86.67 c	87.63 c
	±1.05	±1.69	±0.46	±1.06
Match	7.60 d	83.34 d	84.80 d	85.46 d
	±0.91	±0.67	±0.95	±1.15
Saimix	6.22 e	81.79 e	83.50 e	83.95 e
	±0.60	±0.79	±0.93	±0.54
Avant	11.08 b	92.11 b	89.59 b	88.04 b
	±0.91	±1.14	±2.48	±1.84
F. value	1717.8 *	283.034*	883.90 *	4948.17*
L.S.D	2.95	0.837	0.464	0.223

Table 2. Immediate and latend effect of the five compounds against *Spodoptera littoralis* on the sugar beet crop for the season 2013 after different intervals under field conditions.

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Table 3. Control index and relative potency levels of different compounds applied against the
cotton leaf worm, Spodoptera littoralis (Boisd.) during the two seasons.

Insecticides	Rate/ Fadden	The men Red % during two			Control	Potency
			seasons the			level
		season	season	two		
		2012	2013	seasons		
Challenger	50cm ³ /100L				100	1.52
24%EC		96.69	96.07	96.38		
Dimilin	125 cm ³				69.03	1.05
48%EG		66.00	67.07	66.53		
Match	40 gm				67.30	1.02
50% WG		64.44	65.30	64.87		
Saimix	160 cm ³				65.48	1
5%EC		62.36	63.86	63.11		
Avant	50cm ³ /100L				73.02	1.11
15%EC		70.55	70.21	70.38		

* Potency levels were based on Saimix the least effective compound.

Table 4. Changes in the total protein content in S. littoralis larvae treated with five
insecticides during the two seasons (2012-2013).

Insecticides	Season 2012		Season 2013	
	±S.E. Mean	decrease %	±S.E. Mean	decrease %
Challenger	14.83	36.24	17.88	43.72
	±1.30		±1.15	
Dimilin	18.23 ± 0.74	21.64	20.73 ±1.07	34.74
Match	19.966 ± 0.37	14.18	19.40 ±2.75	38.93
Saimix	20.27 ±3.15	12.85	22.19 ±2.08	30.15
Avant	15.61 0.43 ±	32.90	18.05 ±1.44	43.18
Control	23.26 ±0.86	-	31.77 ±1.48	-

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	Season 2012		Season 2013		
Insecticides				n 2013 activity % -35.12	
	±S.E.	activity %	± S.E.	activity %	
	Mean		Mean		
Challenger	29.32**	-36.41		-35.12	
	±2.43		27.32**		
			± 1.28		
Dimilin	113.02 ***	145.10	119.02***	182.64	
	± 2.45		± 8.98		
Match	107.10 **	132.27	110.76**	163.02	
	±7.04		± 5.168		
Saimix	103.78 *	125.07	107.44**	155.14	
	±5.74		± 2.59		
Avant	37.385**	-18.92	35.38**	-15.98	
	± 3.06		± 1.67		
control	46.11	-	42.11	-	
	±1.32		± 1.67		

Table 5. Changes in chitinase activity in *S. littoralis* larvae treated with five insecticides during two Seasons.

Biochemical studies

The data in table (4) showed that the biochemical changes in the total protein content in S. littoralis larvae treated with five insecticides during the two seasons (2012-2013):

The Chalenger and Avants caused more significant decrease in the total content of protein than IGR compounds. Chalanger caused most decrease (36.24 and 43.72) and Avant (32.90 and 43.18) whereas IGR compounds the most of them had a decrease of protein content as Dimilin (21.64 and 34.74) followed by Match (14.18 and 38.93) then Saimex (12.85 and 30.15) compared to the untreated larvae during the two seasons (2012 and 2013).

The data in table (5) showed that the IGR compounds had significant increase in chitinase activity as Dimilin showed higher increase (145.10 and 182.64), followed by Match was (132.27 and 163.02) then the saimex was (125.07 and 155.14), on the other hand the Challenger caused significant decrease in chitinase activity (-36.41 and -35.12) followed by Avant (-18.92 and -15.98) compared to the untreated larvae during the two seasons

DISCUSSION

Challenger has an immediate effect, and this effect is based on the nature of the work of the compound where affect the insects through contact or as stomach poison. It also affects the production centers of energy in the insects and thus leads to its death, so it is a suitable alternative in the programs of integrated pest management (IPM) (Hamdy and Walaa 2013).

The data obtained from tables 1, 2&3 showed that the Challenger gave higher reduction percentage than Dimilin, Match, Saimix and Avant of the *S. littoralis*. after 24 hours in both sessions 2012 and 2013, these results were coincides with the results of Kamaraju et al 2011 and Braham et.al.2012 who stated that Challenger was found effective and has shown a drastic reduction in efficacy after spraying within 24 h exposure in *An. stephensi* and *An. Culicifacies* mosquito.

The lufenuron is an insect growth regulator that interferes with chitin synthesis, disrupt hormonal balance with exchanging in molting process, and inhibit the insect's growth (Oberlander and Silhacek, 1998). (Reda et al 2013) added that the lufenuron was more toxic against the 2nd and 4th larval instars of *S. littoralis* at LC20 and LC50 than mimic and their toxicity increase depending on the concentration and the time after treatment.

Babariya et al. 2010 stated that Avant works by inhibiting sodium ion entry into nerve cells, resulting in paralysis, inhibit propagation of nerve potential and death of targeted pests. The present work showed that the Dimilin, Match, Saimix and Avant had significant increase in reduction percentage of the S. littoralis., after 3,5 and 7 days but less than Challenger, these results were in agreement with results of Zidan et al 2013 who decided that IGIs lufenuron and chlorfluazuron recorded moderate curative ovicidal activity after 48 h. Likewise Babariya et al. 2010 who added that paralysis and death by Avant occur within 48 hours. Insects exposed to it exhibit the following symptoms: feeding cessation, uncoordination and then paralysis these results explain why Avant did not give immediate effect percentage reduction in our results in the present work

These results were coincidence with the results of the present work that revealed that Match and Dimilin more toxic against Avant as seen in table (3)

Sallam, 1999 added that ovicidal activity of the Dimilin could be due to disturbance in cuticle formation of the embryo.

Also data in table (3) included that the comparison on basis of potency levels, the relative potency level can be used as a convenient method in comparing the degree of toxicity of different compounds to any pest, the potency levels of tested compounds are expressed as a number of folds, compared with the least efficient compound included in the evaluation against the tested pest. Hence, the number of folds representing the potency levels was obtained by dividing the mean reduction percent of Saimix (63.11) as a standard compound on other compounds in case of larval of *S. littoralis.* Furthermore the comparison on basis of control index: The control index is a mean for comparing the relative toxicity of compounds (Sun 1950). In comparing the toxic action of the five compounds, Challenger was taken as the standard compound and given the arbitrary index value as 100 units.

The proteins help to synthesize microsomal detoxifying enzymes which assist in the detoxification of toxicants that enter into the insect body. It is the most important components of the biochemical milieu of insect that bind with the foreign compounds. In general, the problem of protein synthesis is intimately related to the metabolism of nucleic acids. (Wilkinson 1976 and Ahmed et al. 1985)

The data in table (4) showed that the Challenger and Avants caused more significant decrease in the total content of protein than IGR compounds during the two seasons (2012 and 2013). These results were in agreement with the results obtained by (Shaukat et al 2012) who reported that Challenger and Avant caused maximum reduction in growth, sporulation and conidial germination by *Isaria fumosorosea* in relation to the control treatment these results explain why challenger and Avant caused more significant decrease in the total content of protein than IGR compounds

Abdel-AaL 2006 added that Chlorfluazuron caused significant decrease of total proteins, lipid and carbohydrates likewise, (Sheble 1979). Also (El Shikh 2002) and (Abdel-all 2003) reported the same results for both total proteins and carbohydrates for *S. littoralis* and A. ipsilon respectively following flufenxorone treatment. The protein pool of the haemolymph functions as a reserve source of protein synthesis needed for growth and pupal life (Florkin and Jeanuiaux, 1964).

The elevations of chitinase were secondary effect of Dimilin, the primary effect involved a block in incorporation of uridine 5'-diphospho-N-acetylglucose- amine into chitin. Chitin synthetase carried out this polymerization step (Verloop 1977). Yu and Terriere 1977 added that the increased chitinase activity by the reduced activity of -- ecdysone metaboliz --enzymes, consequently ecdysone accumu-lation stimulated hyperchitinase activity.

The data in table (5) showed that the IGR compounds had significant increase in the chitinase activity these results were in agreement with the results of (Ishaaya and Casida 1974, Lee et al. 1994, and Abdel-AaL 2006) who reported that the IGR caused increase in chitinasa activity after treatment of housefly larval cuticle, *Hyphantria cunea* and S.L with IGR respectively.

On the other hand the data in table (5) also showed that the Challenger and Avant caused significant reduction in chitinase activity these results were in agreement with Shaukat et.al 2012 who reported that Challenger caused significant reduction in chitinase activities whereas Avant proved to be the safest insecticide causing lowest reduction in enzyme chitinase activities

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