Egypt. J. Plant Prot. Res. Inst. (2019), 2 (4): 734 - 740

Egyptian Journal of Plant Protection Research Institute

www.ejppri.eg.net

Food consumption, utilization and biochemical impacts of some insecticides on the cotton leafworm *Spodoptera littoralis* (Lepidoptera*:* Noctuidae*)*

Abd El Rahman, S.F.; Saleh, H.A. ; El-Gably, A. R. and Yacoub, Sh.S.

Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.

ARTICLE INFO

Article History

Received: 16 / 10 / 2019

Accepted: 21 /12 /2019

Keywords

Spodoptera littoralis, control, insecticides and biochemical impacts .

Abstract:

This experiment was conducted on the 4th instar larvae of *Spodopetra littoralis* (Boisd.) (Lepidoptera*:* Noctuidae) to investigate the impact of some pesticides belonging to different groups in the laboratory to evaluate their antifeedant activity, relative growth rate and utilization of ingested food . Moreover the effect of the tested compounds on the total protein, total carbohydrate, total lipids and acetylcholinesterase were recorded. The obtained results clear antifeedant index for owner 5%EC seems to be the most powerfull tested compounds followed by dimilin 48%SC. Also, the relative growth rate (RGR) of the 4th instar larvae of *S.littoralis* fed on castor leaves treated with owner 5% was decreased clearly often one day exposure followed by strong 30% SC. After three days exposure emafel 45% ME achieved the lowest utilization of ingested food (FCI). Over and above the biochemical studies explained the four tested insecticide represented significant decrease in the amount of total carbohydrate, totalprotein, total lipids and acetylcholinesterase enzyme. Owner 5% caused the highest change percentage in total lipids (45.12%). Whereas, dimilin 48%SC cased the highest change percentage in total protein and total carbohydrate (33.07 and 54.6) respectively. Dimilin was the most inhibitive of acetylcholinesterase activities 28.06 change percentage.

Introduction

Cotton leafworm *Spodoptera littoralis* (Boisd.) *(*Lepidoptera*:* Noctuidae*)* is one of the major pests in Egypt and other countries in Africa and Asia. It causes considerable damage to cotton and other cultivated crops and vegetables causing severe damage and consequently reduction in the obtained yield (Nasr *et al*., 1984; Ahmed, 1988 and Korrat *et al*., 2012) makes it a model of serious polyphagous pests.

Emamectin benzoate (1.9% EC) (Methylamin :Avermactin) belongs to Avermactin group of chemicals produced by the soil-dwelling actinomycete, *Streptomyces avermitilis*. It represented a second generation of abamectin in avermactin family which acts as nerve poisons stimulate (Fritz *et al*., 1979) which block the post synaptic potential of neuro muscular junction leading to paralysis and finally to the death.

Indoxacarb has a good toxin effect as a new class of oxidiazine insecticide against Lepidoptera pest with nearly no effect on non target insects by blocking the movement of sodiumion and cause stop feeding and paralysis (Dinter and Wiles, 2000).

The use of the insect growth regulators (IGR,s) for the control of insects of economic importance have been widely acclaimed, either as juvenile or other compounds (Smagghe *et al.,* 1995), these compounds interfere with the normal growth for development of insects and their effect could extended to affect the insects reproduction potential as well as other effects on the physiology of treated insects (Abdel-Aziz, 2012).

The aim of this work is to present the evaluation of the effect of owner 5% EC, dimilin 48% SC, emafel 4% ME and strong 30% SC on the feeding activity and food consumption and utilization of *S. littoralis*. Also, the evaluation of the latent effect of the formercompounds on the total carbohydrate, total protein, total lipids and acetylcholinesterase activity as main components of insects was conducted.

Materials and methods

1. Rearing of *Spodopetra littoralis* :

Collected egg-masses of *S.littoralis* from the field were allowed to hatch and the larvae were fed on fresh leaves of castor bean. The rearing was carried out under laboratory conditions 27cº and 55-65 % RH. The 4th instar larvae were selected on the basis of weight. The chosen larvae were starved for about 4 hours before feeding on leaves of castor bean which were treated by the followed compounds by using dipping method. The remaining living larvae were allowed to fed on castor bean leaves until the pupation period and emergence.

2.Insecticides used:

2.1.Lufenuron (Owner 5% EC) producd by: El-moneer for Agricultural .

2.2.Diflubenzuron (Dimilin 48%) producd by: Eristia life co.

2.3.Emamectin benzoate (Emafel 4% ME) producd by: Al-Qawafel Technical Ind. Agr.Co.

2.4.Indxocarb (Strong 30% SC)producd by: Spire for Agricultural.

3. Effect of compounds on food consumption and utilization:

The effect ofthe former compounds on the food consumption and utilization by the fourth instar larvae was investigated . one hundred larvae (4th instar) of *S.littoralis* was starved 4hours and then weighed fresh castor bean leaves, *Ricinus communis* were weighed then leaves were dipped for 10 second in the different compound solution . The treated leaves were left in shade to air dried. Twenty leaves for each treatment plus control treatment were divided into 4 replicates each one with 5 larvae each kept in glass containers with treated leaves. Another 4 replicates of larvae were kept in similar containers with untreated leaves as check .the larvae were daily individually weighed for 5day. The amount of consumed food was calculated, the antifeedant index (AFI) was calculated from the formula of Sadek (2003).

AFI = [(C - T) / (C + T)] x 100 according to

C: Food consumption of control leaves

T: Food consumption of treated leaves

Also, the faces were weighed and consumed was determined. The nutritional indices of growth rate (RGR) were calculated by Farra *et al*. (1989) as follows :

Relation growth rate (RGR) = ΔB/BaT.

Efficacy of conversion of ingested food (ECI) = (ΔB / I) x100

Where:

I: Weight of food consumed .

Ba: Mean of insect weight during the experiment.

T: Feeding period in day.

ΔB: Chang in body weight.

F: Weight of faces produced during the feeding period.

Data were subjected to analysis of variance (ANOVA), (F test) and the least significant differences (LSD) were calculated (Litchfield and Willcoxon, 1949).

4. Biochemical effect :

4.1. Sample preparation:

Fifteen to twenty treated larvae were homogenized in achilled glass Teflon tissue homogenizer (ST-2 Mechanic-preczyina, Poland). Homogenates were centrifuged at 8000 r.p.m. for 15 min at 2 ºC in a refrigerated centrifuge. Supernatants were kept in a deep freezer at -20ºc till use for biochemical assays. Double beam ultraviolet/visible spectrophotometer was used to measure absorbance of colored substances or metabolic compounds. Which is referred as enzyme extract, can be stored at least one week without appreciable loss of activity when stored at 5°C.

4.2.Total carbohydrates:

Total carbohydrates were estimated in acid extract of sample by the phenol-sulphuric acid reaction of Dubios *et al.* (1956). Total carbohydrates were extracted and prepared for assay according to Crompton and Birt (1967). Sample (1 gm) was homogenized in 0.3N HClO4 (5 ml) at ºC for 1 min. The homogenate was kept in ice for further 10 min. Insoluble matter was removed by centrifugation for 3 min.at 2000 r.p.m. and washed twice in ice–cold HClO4 (5ml) by redispertion and centrifugation. The three supernatant combined into acid extract. Hundred microliters of the acid extract were added into a colorimetric tube to 0.5 ml of phenol (20 percent w/v). Then 5 ml of concentrated sulfuric acid were added rapidly with shaking .

The tubes were allowed to stand 10 min, then they were shaken and placed for 10-20 min in water bath at 25 to 30 ºC before readings. Blanks were prepared by substituting distilled water for the sugar solution. The absorbance of characteristic yellow –orange color is measured at 490 nm against blank. Total carbohydrate is expressed as : µg glucose / gm fresh weight.

4.3.Total proteins:

Total proteins were determined by the method of Bradford (I976). Protein reagent was prepared by dissolving 100mg of Coomassie Brilliant blue G-250 in 50ml 95% ethanol. To this solution 100ml 85% (W/V) phosphoric acid were added . The resulting solution was diluted to a final volume of 1 liter. Sample solution (50µl)or for preparation of standard curve 50µl of serial concentrations containing 10 to 100µg bovine serum albumin were pipetted into test tubes . the volume in the test tube was adjusted to 1 ml with phosphate buffer (0.1M, pH 6.6). Five millimeters of protein reagent were added to test tube and the contents were mixed either by inversion or vortexing. The absorbance at 595 nm was measured after 2 min and before 1 hr against blank prepared from 1 ml of phosphate buffer and 5 ml protein reagent. Total proteins was expressed as mg/g-b-wt.

4.4.Total lipids :

Total lipids were estimated by the method of Knight *et al*. (1972) using phosphovanillin reagent prepared by dissolving of 0.6 gm pure vanillin in 10 ml ethanol and completed to 100 ml with distilled water.Then 400 ml conc. Phosphoric acid were added .

4.5.Procedure :

250 ul of sample were added to conc. sulphuric acid (5 ml) in a test tube and heated in aboiling water bath for 10 min . After cooling to room temperature , the digest was added to phosphovanillin reagent (6 ml). After 45 min , the developed color was measured at 525 nm against reagent blank .Optical density was compared to that of a reference standard and results expressed as mg lipids/ ml heamolymph .

4.6.Acetylcholinesterase activity:

The reaction mixture contained 200 µl enzyme solution , 0.5 ml 0.067 M phosphate buffer (pH7) and 0.5 ml acetylcholine bromide (3 mM). The test tubes were incubated at 37 ºc for exactly 30 min. 1 ml of alkaline hydroxylamine (equal volume of 2 M hydroxylamine chloride and 3.5 M NaOH) was added to the test tubes . Then 0.5 ml of Hcl (1 part of conc. Hcl and 2 parts of ΔH_2O) was added. The mixture shaken vigorously and allowed to stand for 2 min. 0.5 ml of ferric chloride solution (0.9 M $Fecl_3$ in 0.1M

Hcl) was added and mixed well. The decrease in acetylcholine bromide resulting from hydrolysis by acetylcholinesterase (AchE) was read at 515 nm .

5. Statistical analysis :

All experiments contained 3-4 replicates (insects homogenates), and the results of biochemical determinations were pooled from triplicate determinations. The results were analyzed by one-way analysis of variance (ANOVA) using Costat statistical software (Cohort software , Berkeley). When the ANOVA statistics were significant ($P < 0.01$), means were compared by the Duncan's multiple range test.

Results and Discussion

1. Estimation of antifeedant index value of different formulation:

Antifeedant effects and toxicity of owner 5% EC , dimilin 48% SC, emafel 4% ME and strong 30% SC, formulations at 0.25, 0.125and 0.0625 field recommended rates against 4th *S.littoralis* instar larvae were estimated and calculated after 24, 48 and 72 hours from the beginning of the experiment though the consumption of tested leaner of castor been leaves Table (1).

Table (1): Effect of different concentration of owner , dimilin, emafel and strong on antifeedant activity of 4th instar larvae of *Spodoptera littoralis*.

Treatments	Does (Field/rate)	Antifeedant index (AFI) % Time exposure(day)			Mean
		1	2	3	
Owner	0.0625	25.6	41.2	64.8	43.9
	0.125	47.1	65.6	71.7	61.5
	0.25	56.2	69.5	72.3	66.0
Dimilin	0.0625	20.6	31.7	52.9	35.1
	0.125	40.5	44.3	56.8	47.2
	0.25	50.2	56.5	60.8	55.8
Emafel	0.0625	14.6	27.3	61.3	34.4
	0.125	20.6	34.9	65.2	40.2
	0.25	29.2	44.9	66.5	46.9
Strong	**0.0625**	**18.1**	**24.7**	**39.1**	27.3
	0.125	26.1	30.7	42.4	33.1
	0.25	31.2	39.4	45.1	38.6

The obtained data, clearly, indicated AFI values increased with the increase of tested concentration material. The rate of increase was recorded by elapse of time after treatments showing 18.1-56.2, 247-69.5 and 39.1-72.3 % for 1, 2 and 3 days, respectively. The greatest antifeedant were achieved by owner at 0.25 field rate being 56.2, 69.5 and 72.3% after elapsed time 1,2 and 3 days, respectively.

On the contrary, strong at 0.0625 field /rate had the lowest antifeedant value among all formulation acting 18.1, 24.7 and 39.1% after 1,2 and 3 days, respectively.

2. Food consumption and utilization:

At the same conditions, the treatments owner, dimilin , emafel and strong reduced the nutritional indices RGR and ECI of the fourth instar larvae. The data presented in Table (2) clearly showed that the majority of previous insecticide treatments decreased the relation growth rate (Food consumption) and also, the efficiency of conversion of ingested food (food utilization) of 4th instar *S. littoralis* larvae. The ECI and RGR were reduced by the high concentration and increased with increasing the time elapsed of the treatment Table (2) these results were agreement with those obtained by Barrania (2013) who found that feeding the 4th instar *S. littoralis* larvae on treated cotton leaves at different rates of the lost compounds decreased the food consumption. Also the relative growth rate of 4th instar larvae of *S. littoralis* fed on cotton leaves treated with chlorantraniliprole , thiamethoxam and

novaluron at 1,1/2 and 41/4 field rates was decreased.

Table(2): The effect of different treatments on nutitional indices,related by food consumption and utilization of the 4th instar *S.littoralis* larvae.

Nutritionnal indices	Feeding period	Relative growth rate (RGR) and efficiency of conversion of ingested food (ECI)Mg/gm												
		control	Owner			Dimilin			Emafel			Strong		
			0.0625	0.125	0.25	0.0625	0.125	0.25	0.0625	0.125	0.25	0.0625	0.125	0.25
RGR	1day	0.056	0.63	0.701	0.6	0.644	0.801	0.6	0.79	0.7	0.631	0.526	0.537	0.5
	2day	0.058	0.547	0.593	0.513	0.206	0.331	0.271	0.632	0.601	0.538	0.049	0.108	0.213
	3day	0.193	0.172	0.181	0.151	0.201	0.31	0.27	0.259	0.231	0.195	0.049	0.041	0.037
	4day	0.201	0.02	0.029	0.017	0.188	0.201	0.199	0.232	0.2	0.193	0.033	0.039	0.013
	5day	0.656	0.008	0.011	0.004	0.036	0.152	0.1	0.037	0.023	0.029	0.027	0.036	0.003
ECI	1day	19.41	20.75	23.81	9.81	42.5	49.81	33.21	37.291	36.361	31.311	41	39.39	31.3
	2day	20.86	20.32	22.7	18.55	39.67	41.61	30.91	14.997	14.011	13.691	13.75	14.81	19.47
	3day	22.42	11.91	17.11	11.91	21.82	24.71	18.75	8.997	10.321	8.673	13.48	14	13.48
	4day	26.32	10.96	15.71	8.88	17.69	20.83	15.52	5.271	3.316	3.711	13.44	13.21	7
	5day	68.51	9.88	14.19	7.91	8.72	11.01	7.33	3.289	2.217	2.001	12.28	12.11	5.12

3.Biochemical effects:

3.1. Effects on total carbohydrates, total protein, total lipids and acetylcholine esterase:

3.1.1.Effects on total carbohydrates:

Data in Table (3) indicated that emafel gave the highest decrease in the total carbohydrate activity lower than control, were 45.6%.in the owner was recorded the lowest decrease, being 3.19% . According to activity ratio represented in the same table, the obtained values 0.54, 0.62, 0.71 and 0.97 time less for emafel, dimilin, strong and owner with the control value , respectively.

3.1.2.Effect on total proteins :

In Table (3) showed that a significant changes in the activity of total protein resulted from the treated 4th instar larvae Emafel and Owner caused significant reduction than control were 33.07 and 24.99%, respectively .but ,it was obvious that there was no significant different between the efficacy of dimilin treatment on the total protein activity was 2.49% than control. Depending on the activity ratio as shown in Table (3) emafel was 0.69 but the activity ratio of owner, strong and dimilin were 0.77, 0.86 and 1.03, respectively.

3.1.3. Effect on total lipids:

Data in Table (3) indicated that owner gave the highest decrease in the total lipid activity lower the control,was 45.12%. While, the remaining treatments were recorded lower decrease than control, they gave 23.75,8.29 and 7.71% change by dimilin, emafel and strong the control which cased 6.87 as mean of total lipids. According to activity ratio represented in Table (3) the obtained value 0.55, 0.76, 0.92 and 0.92 the less for owner, dimilin, emafel and strong with the control, respectively.

All used compounds were caused significantly decrease in the total carbohydrate, total protein and total lipids. There results are in harmony with those obtained by Anwar and Abdel-Mageed (2005) who found that reduction in carbohydrate content, total protein and total lipid of the *S.littoralis* when treated with castor oil, gossypol, Diflubenzuron, tebufenozide, hexaflunuron ,flufenoxuron ,chlorfuazuro and lufenuron. Also, these results are in agreement with Abdel-Salam *et al.* (2018) they found the effect of protecto, viruset ,cascade and ataborn caused significant decrease in the amount of total protein ,total carbohydrate and total lipids in 4thinster of *S. littoralis* larvae.

3.1.4. Effects of acetylcholinesterase activity:

Table (3) showed AchE activity in the 4thinster of *S.littoralis.* The obtained results indicated that. dimilin caused the highest significant decrease in AchE enzyme activity

(28.06%) reduction than control. In the contrary strong was caused the little increase in the AchE enzyme activity by 11.8% than control, the remaining treatment were achieved decreasing than control as 15.99 and 8.45% by owner and emafel, respectively.

Depending on the activity ratio value dimilin was the highest decrease (0.72 time) less than control while, the remaining treatments obtained values 0.92, 0.88 and 0.84 time less for emafel, strong and owner,respectively .

Table(3): Means of total lipids, total proteins, total carbohydrates and acetylcholinesterase percentage change and activity ratio in 4th instar larvae of *Spodoptera littoralis* after treatments by tested compounds .

Treatment	Acetylcholinesterase			Total lipids			Total proteins			Total carbohy		
	Mean ± SE (gm/g.b.wt.)	Change %	Activity ratio	Mean ± SE (gm/g.b.wt.)	Change%	Activity ratio	Mean ± SE (gm/g.b.wt.)	Change %	Activity ratio	Mean ± SE (gm/g.b.wt.)	Change %	Activity ratio
Owner	353±3.51 $_{b}$	15.99	0.84	3.77±0.15 $_{c}$	45.12	0.55	31±0.64 $_{bc}$	24.99	0.77	22.46±0.73 $_{a}$	3.19	0.97
Dimilin	302.3±4.33 $_{c}$	28.06	0.72	5.24±0.18 $_{b}$	23.73	0.76	40.3±1.17 $_{a}$	2.49	1.03	14.5±0.76 $_{bc}$	37.5	0.625
Emafel	384.67±7.86 $_{a}$	8.459	0.92	6.3±0.19 $_{a}$	8.29	0.92	27.66±0.73 $_{c}$	33.07	0.69	12.6±0.19 $_{c}$	54.6	0.54
Strong	370.67±6.06 $_{ab}$	11.89	0.88	6.34±0.18 $_{a}$	7.71	0.92	34.56±0.59 $_{b}$	19.85	0.86	16.53±0.80 $_{b}$	28.75	0.71
Control	420.22±9.28 $_{c}$			6.87±0.22 $_{a}$			41.33±1.30 $_{a}$			23.2±0.95 $_{a}$		
F.value	37.01	----		49.58	----		39.85	----		41.76	----	
L.S.D.	29.44			0.815			4.17			3.29		

References

Abd El-Salam , A. H.; Mabrouk, A. M. ; Eel-Serafy, H. A. K. and Faiz, N.M. (2018): Impact of some bioagent and insect growth regulator to some biochemical aspects of the cotton leafworm *Spodoptera littoralis* (Boisd.). Egypt. J. Agric. Res., 96(4):1361-1368.

Abdel-Aziz, M.M. (2012): Evaluating effecting of certain bioagents and insect growth regulator against the cotton leafworm *Spodoptera littoralis* using molecular technique Ph.D. Thesis Ain Shams Universty.

Ahmed, T.R.(1988): Field studies on pheromone trapping of cotton leafworm *Spodoptera littoralis* (Boisd.) (Lep.noctuidae). J. App. Ent.,105 : 212-215.

Anwar, E. M. and Abd El-Mageed A. M. (2005): Toxicity impacts of certain insect growth regulators on some biochemical activities of the cotton leafworm. Egypt. J. Agric. Res., 87(2) :915 -935 .

Barrania, A. A. (2013): Antifeedant, growth inhibitory and toxicity effects of chlorantramliprole ,thiamethoxan and novaluron against the cotton leafworm *Spodoptera littoralis* (Bioisd.) (Lepidoptera:Noctuidae) in cotton fields. Egypt J. Agric. Res, 91 (3) :903-911.

Bradford, M.M.(I976):A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principle of protein-dye binding. Anal.Biochem., 72:248-254.

Crompton,M. and Birt , L.M. (1967): Changes in the amounts of carbohydrates, phosphagen and related compounds during the metamorphosis of the blowfly , *Lucilia cuprina.* J. Insect Physiol.,13:1575-1595.

Dinter, A. and Wiles, A. J. (2000): Safety of the new Du pont insecticide indoxacarb to beneficial arthropods an overview. IOBC/WP RS Bulletin, 23:149-156.

Dubios,M.; Gilles,K.A.; Hamilton,J.K.; Rebers,P.A. and Smith,F. (1956): Colorimetric method for determination