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Potency of certain insecticides and their effect on some biochemical aspects using semi-field technique in *Spodoptera littoralis*

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Abstract
The comparative potency of tested insecticides; namely Lamba Val, (Lambda-cyhalothrin), Acetathrin El-Nasr (Acetamprid + Lambda-cyhalothrin) and Tracer 24 % (spinosad) from different chemical groups was evaluated to confirm the most effectual insecticide against *Spodoptera littoralis* larvae. The semi-field applications, showed significant difference effect between 2nd and 4th intstars during tested days. The 2nd instar larvae were more susceptible than 4th instar larvae from the first day of application and gradually increased to reach its maximum effective day after 7 days in case of testing the 2nd instar larvae, while the maximum mortality reached after 10 days in case of 4th instar larvae with all tested insecticides. The most effective insecticide was the mixture of Acetamprid and Lambda cyhalothrin followed by Lambda cyhalothrin. Spinosad was the slowest effectiveness in controlling *S. littoralis*, comparing with Acetamprid + Lambda cyhalothrin and Lambda cyhalothrin. Biochemical studies revealed that both LC_{50} and LC_{25} had highly significant reduction in total protein content and alkaline phosphatase activity in the 4th instar larvae with all tested compounds compare with control. In the same trend, the tested insecticides, were more effective on Na^+ and Ca^{++} by their significant reduction effect, particularly Na^+ with LC_{50}'s and not significant between each other. On the other hand, no significant effect on K^+ was noticed.

Keywords: *S. lilloralis*, protein, ChE, alkaline phosphatase, Na, Ca, K, Acetamiprid, lambda-cyhalothrin, Spinosad

Introduction
The cotton leafworm, *Spodoptera littoralis* (Boisd.) is considered one of the mainly destructive pests for cotton and other vegetables, which the most valuable crops in the country. Larvae was known as a leaf eater accepting almost all herbaceous plants and caused great damage for these crops (Abdel-Wahab, 2002 and Kandil *et al.*, 2003) [6, 28]. Several insecticides and applications were required to control the pest (Abou-Taleb, 2016) [9]. The intensive uses of insecticides led to development of resistance in these pests that leads to failures in control by conventional insecticides in the field (Ahmad and Arif, 2010) [10].
Synthetic pyrethroids aimed to enhance the activity and specificity of the natural pesticide pyrethrum. They are a functionally toxin, in a secondary way causing contrary effects, as a result of neuronal hyper excitability. These are confirmed by the lack of atomopathologic injuries in the central nervous systems, regular post recurring critical intoxications (Parker *et al*., 1985). The pyrethroids show great affinity to Na^+ channels and inducing their toxic effect via changes in the functions of these channels. The binding of pyrethroids to Na^+-channels causes a prolonged channel opening (Narahashi, 1996 and Vijverberg & Van den Bercken, 1990) [35, 50].
The using of recent insecticides group as neonicotinoids with an exclusive mode of action are belonging to the fast-growing class of insecticides in recent pest defense post the conventional insecticide group than other insecticide groups which acting as agonists to the nicotinic acetylcholine receptor (Ahmed and Matsumura 2012 and Sandor *et al.* 2015) [11, 43]. Therefore, lambda-cyhalothrin 10.6 % mixed with Thiamethoxam 14.1% (SC) is a combination of two active ingredients of pyrethroid and neonicotinoids groups. It has highly effective for controlling insect because it contains Lambda-cyhalothrin group 3; non systemic and Thiamethoxam group 4; systemic insecticides with repulsive properties. In addition, contact, stomach actions and long remaining activity with rapid knockdown (Abd-El-Aziz, *et al.*, 2020) [3].
Spinosad, belongs to a new class of polyketide-macrolide insecticides. It is a combination of spinosyns A and D, derived by fermentation from the naturally occurring soil actinomycete, a metabolite of Saccharopolyspora spinosa (Sparks *et al*., 1998), which is currently registered in several countries. Spinosad acts in two unique ways on nicotinic acetylcholine and Gamma-Aminobutyric acid (GABA) receptors. The extensive global testing spinosad provided an efficient control to key pests in copious crops, including vegetables and cotton (Thompson *et al*., 2000; Salgado and Sparks, 2005; Osorio *et al.*, 2008).
The main objective of this study is to investigate, the efficiency of three insecticide; namely lambda-cyhalothrin, acetamprid + lambda-cyhalothrin and spinosad, against the 2nd and 4th instar larvae under semi field conditions and to measure some aspects biochemical on the 6th larval instars of *Spodoptera littoralis* post treatment as 4th instars for 48 hrs.

Material and Methods
Rearing technique of *Spodaptera littoralis.*
The strain of larvae used in the present study were obtained from laboratory colony that was reared on castor bean leaves in the *Spodaptera littoralis* rearing laboratory, Cotton Leafworm Research Department, Plant Protection Research Institute (PPRI), Agricultural Research Center (ARC), which reared under constant laboratory conditions of 25 ± 2°C and 60 ± 5% RH as described by El-Defrawi, *et al*., (1964).

The Tested Compound

Three commercial products belonging to different groups of insecticides were used.

1- Common name: Acetamiprid 1.6% + lambda-cyhalothrin 3% ready-made mixture (4.6% EC), with the trade name is Acetathrin El-Nasr, the application rate is 250 ml/feddan and it is the product of El-Nasr Company for Intermediate Chemicals.
2- Common name: Spinosad (24% SC), the trade name is Tracer, the application rate is 30 ml / 100 L and it is obtained from Dow Agro Sciences.
3- Common name: Lambda-cyhalothrin (5% EC), the trade name is Lamba Val, the application rate is 200 ml /feddan and it is the product of Al-Kadesia for Fertilizers and Chemicals Company.

Bioassay Tests

These tests were carried out on the 4th larval instars of *S. littoralis* using dipping technique. Serial concentrations of each insecticides were prepared. Castor leaves were dipped for 5 sec. in each concentration according to Abo El-Ghar *et al*., (1994) [8]. Control was dipped in distilled water. Then treated and untreated leaves were left to dry for about 1 hr under room conditions. The 4th larval instar was starved for about 6 hrs then fed for 48 hrs on the treated leaves after that transferred to fresh untreated leaves for five days. Three replicates for all treatments and control were used, with twenty larvae in each replicate. Concentration-mortality percentages were calculated daily and corrected according to Abbott's equation (Abbott, 1925) [1]. LC_{50} & LC_{25} values were calculated by using the probit- analysis method of Finney (1971) [25].

Semi Field studies

Field experiments were carried out at Ramla village, Banha District, Qalyubia Governorate, during season of 2020, on Sweet potato plants, *Ipomoea batatas*. Complete randomized blocks design was used in the area of 6 carats (1050 m^2), which divided into four parts, three for treated and one for control. Each part divided into 3 replicates, spraying of insecticide was done with the recommended rates of the tested insecticides by using a knapsack sprayer on October 10th. Samples of Sweet potato leaves collected directly after spraying within about one hr. on randomly for ten sequential days. Then the treated and untreated Sweet potato leaves were transferred to the laboratory to feeding separate sets of second or fourth larval instars of the cotton leafworm (Abd El-Aziz, *et al.*, 2017) [1]. Each treatment including control, one hundred freshly molted 2nd or 4th instar larvae divided into 5 replicates (each replicate contains 20 larvae). Mortalities were counted after 24 hrs, 3 days, 5 days, 7 days and 10 days from offering treated leaves of Sweet potato with insecticide to larvae.

Biochemical studies

The 4th larval instar of *S. littoralis* treated with LC_{50} and LC_{25} of lambda-cyhalothrin, acetamprid + lambda-cyhalothrin ready-made mixture and spinosad using dipping technique of caster bean leaves Abo El-Ghar *et al.* (1994) [8] and feeding for 48 hrs.; another group of larvae were fed on untreated leaves (dipped in distilled water) were used in this study. The survived healthy 6th instars larvae were collected from the surviving treated as well as untreated larvae after 6 days of treatment for biochemical studies.

Preparation of haemolymph samples for biochemical analysis:

The haemolymph samples were collected from treated and untreated control according to Abo El-Ghar *et al.*, (1994) [8], by clipping third pair of the proleg of abdominal legs with micro-scissors with gentle care to keep the gut from rupturing. Haemolymph was dropped into Eppendorf tubes containing a trace of phenylthiourea to prevent inhibitory effect of tyrosinase (melanization). The collected hemolymph samples were centrifuged at 5,000 rpm for 10 min at 4 °C to pill cell remains and the supernatants were kept at –20 °C until analysis.

Determination of main components

1. **Total Protein Assessment**
   The heamolymph samples of both treated and untreated were used in the biochemicals analysis. Where determined colorimetrically by using kits, total protein purchase from Biodiagnostic Comp, Giza, Egypt, according to the method described by (Gornal *et al*., 1949) [26] depending on Biuret reaction.

2. **Sodium (Na^+) and Potassium (K^+) Assessments:**
   Sodium (Na^+) and Potassium (K^+) determined using kits obtained from BioMed Diagnostic Comp, Giza, Egypt and read at wavelength (620-640).

3. **Calcium (Ca^{++}) Assessment**
   The measurement of calcium (Ca^{++}) is based on the formation of color complex between calcium and O-Cresolphtalein in alkaline medium and the color intensity reflect the calcium concentration in the sample using kits purchased from Spinreact Comp, Spain according to Farell, *et al.*, (1984).

4. **Determination of Cholinesterase and Alkaline Phosphatase Activities**
   The activity of cholinesterase determined according to Knedel and Bottger (1967) [29] and alkaline phosphatase according to Belfield and Goldberg (1971) [14] colorimetric methods using kits obtained from Bio-Diagnostic comp. Diagnostic and research reagents, Egypt.

Statistical analysis

The statistical analysis of data on mortality was subjected to Abbott's formula (Abbott, 1925) [1]. Duncan, (1955) [20] Duncan's Test (DMRT) the probability level was determine to compare the differences among some parameter means ($P<0.05$) by Costat system for Windows, Version 6.311, Berkeley, CA, USA, Costat program (2006). The Toxicity index estimated the efficiencies according to Sun (1950) equation as follows:

Toxicity index = LC_{50} of the most effective compound × 100 / LC_{50} of the compound used.

Results and Discussion

Effect of treated sweet potato leaves with the tested compounds on *Spodoptera littoralis* under semi-field conditions

The corrected larval mortality percentage were calculated after 1day, 3 days, 5 days, 7 and 10 days from spray of tested insecticides; lambda-cyhalothrin, acetamprid +

lambda cyhalothrin and Spinosad, in both the second and fourth larval instars of *S. littoralis* by fed on sweet potato leaves which spray with the recommended rates of these insecticides.

With respect to acetamprid and lambda cyhalothrin mixture was more effective with all the tested times, where corrected mortality percentage recorded 75.86, 92.59, 94.44, 96.30 and 96.30% for the second instar and 31.67, 77.19, 85.96, 91.23 and 92.98% for the fourth instar after 1 day, 3 days, 5 days, 7 days and10 days, respectively. While the least effective compound used when the larvae were fed on the treated sweet potato leaves by spinosad especially after 1 days recorded 51.72% and 28.33% for second and fourth instar, respectively (Tables 1& 2).

As observed in Tables (1& 2), the general mean of acetamprid + lambda cyhalothrin was superior insecticide giving 91.10 and 75.81% mortalities for 2nd and 4th larval instars, respectively, while, treated 2nd larval instar with lambda-cyhalothrin and spinosad caused mortality rate 88.98 and 84.79% mortalities, respectively. In the case of the 4th instar larvae the insecticides, lambda-cyhalothrin and spinosad were almost closed of the general mean. This agrees with Nukala, *et al*. (2015) [37] who investigated bio efficacy of nine modern insecticides under field conditions against *S. litura* and revealed that emamectin benzoate, chlorpyriphos, cypermethrin, and chlorantraniliprole were found most effective. The reduction in *A. ypsilon* population was higher with neonictinoide than pyrethroid in the two tested seasons (Abd-El-Aziz *et al.,* 2019) [4] and Abd-El-Aziz *et al.,* (2017) on *S. littoralis.* Evaluate the efficacy of spintor against *P. gossypiella* recording higher reduction in El-Gharbia than in El-Fayoum governorate in larval population, post 6 days of spraying were 71& 86.3 and 79.2 & 82.7, respectively (Abo Bakr and El Zoghby 2016) [7].

Table 1: Corrected larval mortality percentage of the 2nd larval instars of cotton leafworm, *S. littoralis* after treated with the tested compounds under semi-field conditions in Qalyubia Governorate.

Treatments	% Corrected mortality after indicated days					
	1 day	3 days	5 days	7 days	10 days	Mean
Lambda-cyhalothrin	72.41	88.89	94.44	94.44	94.44	88.93
Acetamprid + Lambda cyhalothrin	75.86	92.59	94.44	96.30	96.30	91.10
Spinosad	51.72	90.74	92.59	94.44	94.44	84.79

Table 2: Corrected larval mortality percentage of the 4th larval instars of cotton leafworm, *S. littoralis* after treated with the tested compounds under semi-field conditions in Qalyubia Governorate.

Treatments	% Corrected mortality after indicated days					
	1 day	3 days	5 days	7 days	10 days	Mean
Lambda-cyhalothrin	33.33	61.40	73.68	82.46	82.46	66.67
Acetamprid + Lambda cyhalothrin	31.67	77.19	85.96	91.23	92.98	75.81
Spinosad	28.33	57.89	75.44	84.21	85.96	66.37

Effect of the tested compounds on the 4th instar larvae of *S. littoralis* under laboratory conditions:

Toxicity of the tested insecticides; lambda-cyhalothrin, acetamprid + lambda-cyhalothrin mixture and spinosad is shown in Table (3). Results indicated that, acetamprid + lambda cyhalothrin mixture gave superior toxic efficacy compared to the other tested compounds. The corresponding LC_{50} & LC_{25} values were 1.6 and 0.013ppm; respectively. Also, data revealed that the LC_{50} & LC_{25} values of spinosad (25 ppm & 8.4ppm), respectively are raised sharply compared with other two tested insecticides. Based on the LC_{50} & LC_{25} values acetamprid + lambda cyhalothrin was taken a standard toxicant and givens arbitrary index values as 100 units. The toxicity index of lambda-cyhalothrin and Spinosad recorded 38.1 and 6.4% at LC_{50} level and 4.33 and 0.15% at LC_{25} level as toxic as toxicity of acetamprid + lambda cyhalothrin; respectively, against the 4th instar larvae of *S. littoralis*. The present result is agreed with Thompson and Hutchins (1999) [47], they reported that the toxic effect of spinosad was significantly lower than that of the other chemical insecticide and the degradation of spinosad in the environment occurs mainly by photo and microbial degradation. Also, El-Moursy *et al.* (2000) [22] stated that the latent toxicity of the bioinsecticide Delfin was significantly lower than that of the chemical insecticide pyrethroid. The pests have low susceptibility to Spinosad in the laboratory (Wang *et al.,* 2009) [51]. Comparing with Acetamprid + Lambda cyhalothrin and Lambda cyhalothrin, Spinosad was the slowest effectiveness in controlling *S. littoralis* that may be due to, resistance or relatively slow mode of acting compared with pyrethroid and neonictinoide.

Table 3: LC_{50} and LC_{25} values of three tested compounds against the 4th instar larvae of *S. littoralis*.

Compound	LC_{50} (ppm)	Toxicity index	LC_{25} (ppm)	Toxicity index
Lambda-cyhalothrin	4.2	38.1	0.3	4.33
Acetamprid + Lambda cyhalothrin	1.6	100	0.013	100
Spinosad	25	6.4	8.4	0.15

Sun's toxicity index= LC_{50} of the most toxic compound/ LC_{50} of the tested compound x 100.

Biochemical studies

The effect of LC_{50} and LC_{25} of lambda-cyhalothrin, acetamprid + lambda-cyhalothrin and spinosad were evaluated on some biochemical parameters of the 6th larval instars of *S. littoralis* haemolymph post treatment as 4th instar larvae for 48 hrs.

Cholinesterase activity:

The activity of cholinesterase was studied in *S. littoralis* larvae. As shown in Table (4) data indicated that, the least activity occurred in control (157.57 U/L). At LC_{50}, the highest enzyme activity was recorded in lambda-cyhalothrin with the percentages increase than control, which was 202.93%. On the other hand, all treatments showed a highly significant increase reached to 477.02, 434.01 and 312.80 U/L for lambda-cyhalothrin, acetamprid + lambda-cyhalothrin and spinosad, respectively. The enzymatic activity in control was 157.57 IU/L. there were significant difference between enzymatic activity resulted from lambda-cyhalothrin and that resulted from the other two treatments as well as the control. Whereas, there was not significant difference in the enzymatic activity resulted from acetamprid + lambda-cyhalothrin and Spinosad.

At LC_{25}, the highest enzyme activity was recorded in acetamprid + lambda-cyhalothrin with the increase percentages of 152.85% than control reached. Treatment with lambda-cyhalothrin and acetamprid + lambda-cyhalothrin showed significant increase reached 391 and 398.43 U/L compared to control. Nevertheless, spinosad showed not significant difference.

The nervous system of insect was attacked by Pyrethroids

which influence the sodium channels with blocking them within its opening point throughout inhibition the inactivation of voltage (Casida, 1973) [15]. This blockage led to a significant acetylcholine release at the synapse and overload of neurotransmitters led to loss the sensation in post-synaptic receptors that appear as depressing feedback on the pre-synaptic receptors, and led to insect paralysis followed by death.

All tested insecticides cause severe cholinergic poisoning in insects by stimulating the cholinesterase which hydrolyzes the neurotransmitter acetylcholine. Over motivation of the nervous system caused insect death. These results in harmony observed by Abd-El-Aziz *et al.*, (2020) [2] both neonicotinoid and pyrethroid insecticides had highly significant increase in AchE of *S. littoralis* homogenate larvae. Spinosad acted throughout the excitation of the insect nervous system, which in bend inducing change in nicotinic and GABA – gated ion channels function which led to obligatory tremors and muscle contractions (Thompson, *et al.*,1995). Moussa *et al.*, (2019) highly significant reduction in AChE activity was produced Spinosad or combined with mint oil larval treatment. The nerve impulses was terminated in cholinergic synapse in the nervous system via hydrolyzing the neurotransmitter ACh (Salgado *et al.*, 1998).

Table 4: Effect of LC_{50} and LC_{25} values of the tested insecticides on cholinesterase (ChE) activity on the 6th instar larvae produced from treated 4th instar larvae of *S. littoralis*.

Treatments	Cholinesterase activity (U/L) at indicated LC levels			
	LC_{50} (ppm)		LC_{25} (ppm)	
	Mean ± S. E	Change%*	Mean ± S. E	Change%*
Lambda-cyhalothrin	477.02 ±69.51a	202.73	391.00 ± 88.39a	148.14
Acetamprid + Lambda-cyhalothrin	434.01 ± 73.13ab	175.43	398.43 ± 29.91a	152.85
Spinosad	312.80 ± 43.54b	98.511	245.94 ± 7.11ab	56.08
Control	157.57 ± 14.59c	-	157.57 ± 14.59b	-

Means with the same letter are not significantly different at p <0.05.
* %Increase or decrease than control = ((Treated-Control) / Control) * 100

Alkaline phosphatase activity

Results presented in in Table (5) indicated that the tested insecticides showed highly significant effect on alkaline phosphatase activity of *S. littoralis* larvae in treated compared to control.

Data showed that, the highest alkaline phosphatase activity was shown in case of corresponding treatment with acetamprid + lambda-cyhalothrin which recorded 0.64 and 0.56 IU/L with reduction percentages of 39.76 and 46.91% at both LC_{50} and LC_{25} values, respectively.

At LC_{50} value, all treatments showed a significant decrease reached 0.72, 0.64 and 0.95 IU/L for lambda-cyhalothrin, acetamprid + lambda-cyhalothrin and spinosad, respectively compared control which recorded enzymatic activity 1.06 IU/L. Likewise, at LC_{25}, treatment with the tested insecticides showed a significant reduction percentage related to the control.

Table 5: Effect of LC_{50} and LC_{25} values of the tested insecticides on Alkaline phosphatase activity on the 6th instar larvae produced from treated 4th instar larvae of *S. littoralis*.

Treatments	Alkaline phosphatase (IU/L) at indicated LC levels			
	LC_{50} (ppm)		LC_{25} (ppm)	
	Mean ± S. E	Change%*	Mean ± S. E	Change%*
Lambda-cyhalothrin	0.72 ± 0.005c	-31.53	0.96 ± 0.005b	-9.65
Acetamprid + Lambda cyhalothrin	0.64 ± 0.013d	-39.76	0.56 ± 0.013d	-46.91
Spinosad	0.95 ± 0.021b	-10.34	0.84 ± 0.003c	-20.84
Control	1.06 ± 0.012a	-	1.06 ± 0.012a	-

Means with the same letter are not significantly different at p <0.05.
* %Increase or decrease than control = ((Treated-Control) / Control) * 100

Alkaline phosphatase is an enzyme indicator in the digestive system tissue so its reduction exhibits the poisonous effect via debility of larvae to digest food which require for all the living processes. Our results may confirm with other studies Abd-El-Aziz *et al.*, (2020) [2] neonicotinoids had no-significant inhibition in alkaline phosphatase and a significant stimulating was recorded with pyrethroid in larval homogenates of *S. littoralis*. Also, agree with (Farag, 2015), reported that the reduction in ACP and ALP activities might be related to the action of treatment. This inhibition may be due to the binding between the bio-agent and the phosphatase enzymes. Abd El-Mageed and El-gohary (2006) [5], they reported that treated larvae of *S. littoralis* with Spinosad induced high significant reduction in alkaline phosphatase activity compared to control. Assar *et al.* (2016) using Spinetoram against *S. littoralis* larvae caused a significant reduce in alkaline phosphatase activity compared to control.

Total protein

Results given in Table (6) indicated that all tested insecticides led to significant decrease in total protein which was more obvious with spinosad in both level of LC_{50} and LC_{25} compared with control and not-significant between each other. In case of treatment with LC_{50} value, the total protein content was 3.58, 4.80 and 3.75 mg/ml for lambda-cyhalothrin, acetamprid + lambda-cyhalothrin and spinosad, respectively, which were significant difference compare with control which being 6.62 mg/ml. On the other hand, there was not significant differences between all treatments. Based the change percentage, treatment lambda-cyhalothrin recorded the highest decrease in protein content which was

46% followed by spinosad and acetamprid + lambda-cyhalothrin, with change of 43.41 and 27.55%, respectively. While treatment with LC_{25} value, the total protein content was significantly lower with spinosad which was 4.04 mg/ml compared with other treatments and control. But there are not significant differences between the other compound and control. On the basis of change percentage, spinosad recorded the highest decrease in protein content which was 39.04%. This agrees with Shaaban *et al*. (1985) [44] which reported that total haemolymph protein content of 6th instar larvae of *S. littoralis* decreased after treatment of the 4th larval instar with pyrethroid compounds. The changes in protein content of the infected insects were linked with interruption in the insect structure and enzyme system, which is responsible for protein metabolism and the used insecticides interfering with the protein synthesis (Abd-EL-Aziz *et al.*,2020) [20]. The decrease in the protein content of the haemolymph in the present work might be due to inhibition of DNA and RNA synthesis, as suggested by Mitlin *et al.* (1977) [33] for boll weevils treated with chitin synthesis inhibitors.

Table 6: Effect of LC_{50} and LC_{25} values of the tested insecticides on the total proteins on the 6th instar larvae produced from treated 4th instar larvae of *S. littoralis*.

Treatments	Total proteins (mg/ml) at indicated LC levels			
	LC_{50} (ppm)		LC_{25} (ppm)	
	Mean ± S. E	Change%*	Mean ± S. E	Change%*
Lambda-cyhalothrin	3.58 ± 0.31 b	-46.00	5.41 ± 0.17 ab	-18.22
Acetamprid + Lambda-cyhalothrin	4.80 ± 0.70 b	-27.55	5.36 ± 0.86 ab	-19.11
Spinosad	3.75 ± .12 b	-43.41	4.04 ± 0.19 b	-39.04
Control	6.62 ± 0.15 a	-	6.62 ± 0.15 a	-

Means with the same letter are not significantly different at p <0.05.
* %Increase or decrease than control (%) = ((Treated-Control) / Control) * 100

Sodium (Na^+)

Results summarized in Table (7) showed that all the treatments had highly significant decrease in sodium cation content especially at LC_{50} level compared with control, where sodium content was 115.48, 117.17 and 114.27 mEq/L with the percentages decrease of 47.58, 46.81and 48.13% than control for lambda-cyhalothrin, acetamprid + lambda-cyhalothrin and spinosad, respectively, compared with control was 220.30 mEq/L. Likewise at LC_{25} treatment, sodium content was significantly lower compared with control, where sodium content was 135.48, 152.78 and 149.44 mEq/L for lambda-cyhalothrin, acetamprid + lambda-cyhalothrin and spinosad, the corresponding reduction percentages were 38.5, 30.65 and 32.16%, respectively compared with control.

Table 7: Effect of LC_{50} and LC_{25} values of the tested insecticides on sodium (Na^+) on the 6th instar larvae produced from treated 4th instar larvae of *S. littoralis*.

Treatments	Sodium (Na^+) (mEq/L) at indicated LC levels			
	LC_{50} (ppm)		LC_{25} (ppm)	
	Mean ± S. E	Change%*	Mean ± S. E	Change%*
Lambda-cyhalothrin	115.48 ± 6.67 b	-47.58	135.48 ± 7.28 b	-38.50
Acetamprid + Lambda-cyhalothrin	117.17 ± 2.46 b	-46.81	152.78 ± 12.11 b	-30.65
Spinosad	114.27 ± 6.02 b	-48.13	149.44 ± 9.15 b	-32.16
Control	220.30 ± 13.17 a	-	220.30 ± 13.17 a	-

Means with the same letter are not significantly different at p <0.05.
* %Increase or decrease than control (%) = ((Treated-Control) / Control) * 100

Potassium (K^+)

Data illustrated in Table (8) showed decrease percentage than control in all treatments with values 18.33, 9.89 and 14.36% at LC_{50}; 14.80, 3.69 and 5.60% at LC_{25} for lambda-cyhalothrin, acetamprid + lambda-cyhalothrin and spinosad, respectively. In addition to a slight increase was recorded of values at LC_{25} values compared at LC_{50} values (where, at LC_{25} values potassium content were 7, 7.92 and 7.76 mEq/L; $LC_{50,}$ 6.71, 7.41 and 7.04 mEq/L; for lambda-cyhalothrin, acetamprid + lambda-cyhalothrin and spinosad, respectively). Nevertheless, non-significant difference in potassium content between treated and untreated larvae of *S. littoralis*.

Table 8: Effect of LC_{50} and LC_{25} values of the tested insecticides on potassium (K^+) on the 6th instar larvae produced from treated 4th instar larvae of *S. littoralis*.

Treatments	Potassium (K^+) (mEq/L) at indicated LC levels			
	LC_{50} (ppm)		LC_{25} (ppm)	
	Mean ± S. E	Change%*	Mean ± S. E	Change%*
Lambda-cyhalothrin	6.71 ± 0.35 a	-18.33	7.00 ± 0.40 a	-14.80
Acetamprid + Lambda-cyhalothrin	7.41 ± 0.49 a	-9.89	7.92 ± 0.90 a	-3.69
Spinosad	7.04 ± 0.48 a	-14.36	7.76 ± 0.80 a	-5.60
Control	8.22 ± 0.73 a	-	8.22 ± 0.73 a	-

Means with the same letter are not significantly different at p <0.05.
* %Increase or decrease than control = ((Treated-Control) / Control) * 100

Calcium (Ca^{++})

Calcium composition in treated *S. littoralis* larvae compared to control are shown in Table (9). All tested insecticides had different effect on calcium which showed highly significant reduction between treated and untreated larvae. At level LC_{50}, all treatments showed a significant decrease reached 1.29, 2.82 and 3.84 mg/dl for lambda-cyhalothrin, acetamprid + lambda-cyhalothrin and spinosad, respectively compared control reached 5.14 mg/dl. On the other hand, lambda-cyhalothrin gave the highest percentage of reduction than control which reached 74.84% followed by acetamprid + lambda-cyhalothrin (45.20%) and finally spinosad (25.29%).

At LC_{25}, treatment with lambda-cyhalothrin and acetamprid + lambda-cyhalothrin are shown a significant reduces reach 2.02 and 3.31 mg/dl with reduction percentages than control reach 60.77 and 35.60%, respectively. While these were not significant difference between spinosad treatment and control which exhibited 4.29 and 5.14 mg/dl, respectively. Notwithstanding, the percentage decrease than control at spinosad reached 16.54%.

Table 9: Effect of LC_{50} and LC_{25} values of the tested insecticides on Calcium (Ca^{++}) on the 6th instar larvae produced from treated 4th instar larvae of *S. littoralis*.

Treatments	Calcium (Ca^{++}) (mg/dl) at indicated LC levels			
	LC_{50} (ppm)		LC_{25} (ppm)	
	Mean ± S. E	Change%*	Mean ± S. E	Change%*
Lambda-cyhalothrin	1.29 + 016 ^{d}	-74.84	2.02 + 0.32 ^{c}	-60.77
Acetamprid + Lambda-cyhalothrin	2.82 + 0.34 ^{c}	-45.20	3.31 + 0.40 ^{b}	-35.60
Spinosad	3.84 + 0.19 ^{b}	-25.29	4.29 + 0.40 ^{ab}	-16.54
Control	5.14 + 0.20 ^{a}	-	5.14 + 0.20 ^{a}	-

Means with the same letter are not significantly different at $p<0.05$.

* %Increase or decrease than control = ((Treated-Control) / Control) * 100

Many studies occurred on proteins, lipids, carbohydrates and other components and their critical role in living cells. While little studies cared about the function of some element Na^{+}, Ca^{++}, K^{+} and their role in the different biological and detoxification process in insect. Calcium of eukaryotic cells, concerned in the management of several cellular activities. It acts an essential role in controlling the important physiological activities due to its leaky channel which considered the most important second messengers not only a charge carrier in muscle contraction and neurotransmitter relief. Therefore, pumps and calcium channels proposed as target sites of novel insecticide as mentioned with (Mink, 2010 and Lümmen, 2013) [32, 30]. Also, gene regulation, membrane transport processes, hormone biosynthesis and cell-death. Calcium ion has some unique properties compare with other, where joins faster to both inorganic and organic anions due to its varying level of hydration and easily form almost insoluble salts with phosphates. It is possible that this will be risky during the development of a cellular energy upkeep chemistry that relies on phosphorylation. Phosphorylation at the expense of adenosine triphosphate (ATP) induces a conformational change to exposes the calcium to the extracellular medium (Wang *et al.*, 2011 and Lümmen, 2013) [52, 30].

In insects and mammals, the storage intracellular calcium sections include mainly the endoplasmic or sarcoplasmic reticulum which found to control the contraction level in a joint way with Na/ Ca exchanger. The muscles contraction of the dorsal vessel energies the circulation of the hemolymph in Drosophila is controlled by calcium like the heart contraction, neuromuscular synapses and central nervous system in mammals (Arnon *et al.*, 1997 and Desai-Shah *et al.*, 2010) [12, 19] which basis for calcium binding is similar in both proteins (Iwamoto *et al.*, 2007) [27]. There is a relation between Na, Ca_2 and the disturbance in the heart function that noticed by (Noble and Herchuelz, 2007; Lümmen, 2013) [36, 30] where, three Na ions are in use/ one Ca_2 meaning that Na exchange Ca activity is electrogenic in particular biological situation, throughout the heart systole with great Na entrance via voltage-gated sodium channels, Na/Ca_2 exchanger be able to operate in the reverse mode justify controlled entrance of Ca_2 into the cell. Also, the Na/Ca_2 exchanger carry calcium to the extracellular place. In mammalian heart muscles, it removes more than 70% of the cytosolic calcium, and in skeletal muscles (McLennan and Kranias, 2004) [31].

The reduction in protein may be affected, thus cells need to regulate the Ca, Na, K, phosphatase, and protein concentrations. The disturbance of them may be cleared as the relation between protein and calcium. Proteins that separate the ion and membrane pumps progressed, this pump calcium out of the cell or into intracellular storage parts. Calcium-handling proteins are taken over new roles to control the intracellular calcium concentrations in the cytoplasm compartments to keep free calcium in low intracellular concentrations, as parts of regulatory circuits, calcium- conducting transmembrane channels resolve closely controlled calcium entry (Clapham, 1995) [16].

Voltage-gated calcium channels facilitate a partial calcium entrance, then a huge calcium relief by transition pores permeability (Ruiz-Meana *et al.*, 2010 and Lümmen, 2013) [40, 30]. Transient Receptor Potential channels are lacked the positively charged voltage-sensor residues, which is a characteristic of voltage-gated Na^{+}-, K^{+}-, and Ca^{++}-channels and involved in several cellular processes as cardiovascular regulation, pressure, smooth muscle tone, inflammation, salivary fluid secretion, cell adhesion, growth control, differentiation and cell death principally by changing membrane voltage and Ca^{++} in the translation of a various of visual and neuronal signals, thus acting functions in sensory process (Damann *et al.*, 2008 and Minke, 2010) [18, 32].

So, it can be improved that compounds disturbing calcium, homeostasis and Na/Ca_2 exchanger in the heart function would be efficient insecticides due to the vital role that calcium acting particularly in emotional cells. These compounds would be highly efficient by agonistically activation of calcium-signaling flows or by blocking negative regulatory loops (Lümmen, 2013) [30].

However, there no insecticides pointing Na/Ca_2 exchanger described no more. It can be expected that inhibition of

insect Na /Ca exchanger in the onward mode must lead to contraction, cramping, paralysis and destruction of neurotransmission, or neuronal degeneration. In spite of this, it would be revealed that many problems in aware the function and structure of insect calcium channel complexes and its relation to various elements even need to resolve.

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