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Field evaluation and some physiological studies of certain insecticides against black cutworm, *Agrotis ipsilon* (Hufn.) larvae

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Abstract
The effect of the recommended field as well as medium field rates of the readymade insecticides, Acetamiprid+ Lambda-cyhalothrin and Lambda-cyhalothrin to control *Agrotis ipsilon* larvae was evaluated. The insecticides showed much higher efficacy when used as poison baits. During February 2020, both insecticides field rates were applied in El-Giza and El-Behaira Governments, at recommended field rates, when appearance the symptoms of infection by cutworms in the potato crop which being 10 larvae, either cutting or damage plant per 25 plants. These insecticides belonging to, synthetic pyrethroids and neonicotinoids groups. The data were collected by counting the number of plants damaged or the eaten plant parts by *A. ipsilon* larvae just before and after three days from the application. The results cleared that there is no significant difference between the two tested Governments, whereas these was significantly difference between each other and control. Acetamiprid + Lambda-cyhalothrin showed in both Governments more effective than Lambda-cyhalothrin.

The effect of sub-lethal field rate of both insecticides had similar effect on each Ache, trehalase, acid and alkaline phosphatase whereas these insecticides showed different effect on total protein, total lipids, invertase, amylase production. Both treated larvae showed highly significant stimulation in AchE and trehalase, while non-significant of amylase synthesis in case of Acetamiprid + Lambda-cyhalothrin treatment. In addition, to total protein, total lipids, invertase for Lambda-cyhalothrin treatments. On contrast, both insecticides caused highly significant inhibition in acid and alkaline phosphatase synthesis, in addition to total protein, total lipids, invertase in case of Acetamiprid + Lambda-cyhalothrin and non-significant in amylase production in case of Lambda-cyhalothrin treatments.

Keywords: Pyrethroids, acetamiprid, lambda cyhalothrin, digestive enzymes, AchE, total protein, lipids

Introduction

Black cutworm (Lepidoptera: Noctuidae) is omnipresent in various countries around the world. It is one of the most destructive and serious underground pests that feed on many parts of plant crops including buds, fruiting points, or leaves of several agricultural crops, tomatoes, corn, potatoes crops and others. The larvae attracted to young growing plant especially with early four pairs of leaves. That led to destroy the crop and the yield reduced (Hill, 1983 and Abd-El-Aziz, *et al*., 2019) [24, 1]. Therefore, controlling it needs to prevent any damage occurs by this pest during the growing time help to increase the yield. Some pesticidal groups, synthetic pyrethroids as Lambda-cyhalothrin (non-systemic), neonicotinoids as Acetamiprid + lambda- cyhalothrin and other groups used mainly to protect the plant crops from cutworms attack. The neonicotinoids groups are the fastest growing group in modern pest protection, which followed the conventional pesticide groups with a unique mode of action by acting as the agonists to the nicotinic acetylcholine receptor in comparative with other pesticide groups (Sandor *et al*. 2015). [34] The using of sub-lethal rate of insecticides has a strong impression on the behavior and physiology of insects and could be monitored to prevent the incident of hurtful impacts of them on the environment. The extensive usage of the same insecticide groups against the same pest may associate with increase insect resistance to these insecticides. Therefore, the target of this work tries to focus on the field evaluation of Lambda-cyhalothrin and Acetamiprid+Lambda cyhalothrin, toward *A. ipsilon* larvae. Then the effect of sub-lethal- field rates, where the larvae not dying as their affect on various physiological processes by it's interfering with the chemical substitute and/or the metabolic enzymes activities (Haynes, 1988). [22] So, it may interest to survey the changes in the different biochemical aspects throughout the poisoning course of insecticide as total proteins content, total lipids, some enzymes activity AchE, trehalase, amylase, invertase, alkaline and acid phosphatase as part of integrated pest management for cutworm.

Materials and Methods

Rearing insects

The *Agrotis ipsilon* larvae; (field strain) were collected from El-Behaira Government and transferred to Department of cotton leafworm, Plant Protection Research Institute, Ministry of Agriculture, Dokki, Giza, Egypt. The larvae reared on castor bean leaves, *Ricinus communis* under laboratory conditions at 22 ± 2 °C and 50 – 60% RH, without any exposure to insecticides according to Abdin, (1979) method; with some modification, according to Abdou and Abdel-Hakim (2017) [6] and Abd-El-Aziz *et al.*, (2019)[1]. The larvae reared individually to avoid cannibalism for many generations until obtained sufficient number of larvae to carry out the experiments for biochemistry assays.

Insecticides used

The readymade insecticides, Acetamiprid + Lambda cyhalothrin (4.6% EC) as Acetathrin El Nasr obtained from El Nasr for Intermediate Chemicals Co. with field rate 250 ml /feddan and Lambda-cyhalothrin (5% EC) is a synthetic pyrethroid as lamba val obtained from Al-Kadesia for Fertilizers and Chemicals Co. with field rate 200 ml /feddan. These are commercial pesticides belonging to different groups were used.

Baits preparation

The baits were prepared by mixing 25 kg of wheat bran with 8-10 litter of water, 1 kg molasses (black honey) as attractive substance was added and mix well and left in worm place over night in dark until fermentation according to Balevski, *et al*.,(1974) [9] with some modification.

Field Application

The field experiments were performed in Kafr kasem village, El Giza and El berka village, El- Behaira, Government during February 2020; experimental area was twelve karats about 2100 m^2 of potatoes crop. The design of experiments was established as complete randomized block by four replicates. The experimental area divided into plots175 m^2 each for treatment, and separated into three parts, the first one as control; baits only, the two remain parts applied at the field rates 250 and 200 ml/25 kg wheat bran for Acetamiprid + Lambda-cyhalothrin 4.6% EC (Acetathrin El Nasr) and Lambda cyhalathrin 5% EC (lamba val), respectively. Each experimental area divided into four-replicates. The field application should be carried out once the symptoms of cutworms appear in the field as the larval populace gets to 10 larvae, damage, or cutting plant / 25 plants. The application time must be before sunset or in the early morning to avoid the harmful effect of UV on the pesticides and make sure the feeding of larvae.

The evaluation of these insecticides occurred by putting a suitable amount of poisoning baits under the potato plants near stem for treatments and non-poisoning baits for control according to protocols of insecticides efficacy against the pest. First once before application and after 3 days post-treatment the number of larvae / plant, the number of cutting, symptoms of damage or feeding leaves of plants was counting randomly in each replicate. In addition, the reduction percentages in the larval populations were calculated according to Henderson and Tilton (1955) [23].

The toxicity experiments

The semi-field technique was done to study the effects of medium field rates 125 and 100 ml / 25 kg wheat bran bait for Acetamiprid+ Lambda-cyhalothrin(Acetathrin Elnasr) and Lambda cyhalathrin (lamba val), respectively. Sub-lethal rates values of each insecticide were incorporated separately with the bait. The starved 4^{th} instar larvae of *A. ipsilon* were fed on the treated baits and another larval groups were fed on baits untreated for three days as control groups. Then, the larvae of treated and untreated collected post three days from treatment and kept frozen at -20 ^{0}C till analysis.

Samples preparation for analysis

The extraction of the whole body of the 4^{th} larval instars samples of treated and untreated *A. ipsilon* homogenized in 0.9% saline solution (1g/ml) using a mortar and a pestle. Using refrigerated centrifuge to centrifuge the larval samples at 5000 rpm for 10 min. The supernatant of each sample reserved in a clean eppendorf tube and kept at -20C^0 in a deep-freezer, three replicates for each until using for biochemical measurement.

Effect of sub-lethal rates on some biochemical parameters in *A. ipsilon* larvae

The biochemical analysis was carried out in Plant Protection Research Institute, Department of Physiology, Dokki, Giza, Egypt. All the tested occurred on the homogenate larvae. Total protein measured colorimetric according to the kits of Biodiagnostic Comp, Giza, Egypt, dependent on Biuret reaction (Gornal *et al.*, 1949) [21].The total lipids as Baronos and Blackstock (1973) [11] method of the phosphovanillin. The acetylcholine esterase activity (AChE) by using acetylcholine bromide (AChBr) as substrate was determined as Simpson *et al.,* (1964) [38] method. The activities of amylase and invertase measured as Ishaaya and Swirski (1970) [26] and Isahaaya *et al.* (1971) [27] by using 3, 5-dinitrosalicylic acid reagent, on purpose of determine the free aldehydic groups of glucose, which formed after digestion of starch or sucrose. In addition, the method of Noelting and Bernfeld (1948) [32] to determine the trehalase enzyme activity.

Statistical analysis

The reduction percentage in field evaluation was calculated according to Henderson and Tilton (1955) [23] equation. All samples of the treated 4^{th} larval instars, homogenates were replicated 3 times, and the statistic analysis of variance was calculated using one-way ANOVA test. Significant differences between treatments using Duncan's test (P<0.05) were determined (Duncan, 1955) [18].

Results and Discussion

Field Application

The field evaluation of Acetamiprid+ lambda cyhalothrin and lambda cyhalothrin was applied at the field rates in El Giza, Kafr kasem village and El berka village, El- Behaira, Governments, on potato crop during February of 2020. Data were collected by counting the number of damage plants, larvae, or plant parts cutting by *Agrotis. ipsilon* larvae before application and post- three days of treatment which represented in (Table 1 and Fig 1). The results cleared that the two insecticides had not-significant difference between two tested Governments but significantly different to each other and control. There are significant reduction in *A. ipsilon* population, the most effective insecticide was Acetamiprid + Lambda-cyhalothrin in El- Behaira Government, where the reduction % were 88.27% followed by 86.79% in El Giza Government. On the same trend Lambda- cyhalothrin was more effective in El- Behaira Government by 85.96% and 84.37% in case of El-Giza Government. Therefore, both insecticides were more effective in El-Behaira than El-Giza Government that may be due to the difference in weather and the susceptibility of *Agrotis.* In addition, Acetamiprid + Lambda-cyhalothrin had significant improved than Lambda-cyhalothrin that may be revealed to it has two active ingredients. Our results are in agreement with early workers on *A. ipsilon* with several insecticides; Abd-El-Aziz *et al.,* (2019) [1] showed a highly reduction in *A. ipsilon* larval population in all plots tested during season 2019 than 2018 with recorded 92.91 and 92.26%, 88.68% by Coragen, Lambda-cyhalothrin + Thiamethoxam and Lambda-cyhalothrin as poison baits, respectively. By the mean of reduction % in the two successive seasons, Anjio (neonicotinoids) had highly effective for the control of *A. ipsilon* than affact power (pyrethroid) because it contains two active ingredients at low rates as effective mixture of two different groups due to a unique mode of action, residual activity which can protect other young plants against harm insects. In addition, Cheng (1984) [15] using of single application of different insecticides is good enough to destruct larvae of *A. ipsilon* at early stage in field. Baxendale *et al.* (2001) [10] evaluated certain pyrethroid included Lambda-cyhalothrin and a non-pyrethroid insecticide where all treatments produced 100% reduction on black cutworm. Cynthia Scott-Dupree, *et al.,* (2007) [17], Lambda-cyhalothrin was more toxic than permethrin for controlling *A. ipsilon* larvae. In addition, the field data during 2002 on *A. ipsilon* larvae showed that the insecticides had significantly different with control while non-significant effect between each other. The lowest mean of infestation (1.3%) in poison bait followed by (1.6%) with Lannate then by (1.8%) in steward while the highest (11.5%) was in the control plot (Muhammad Shaku, *et al.*, 2007) [30]. Sharma (2014) [37] evaluated five insecticides included thiamethoxam against cutworms revealed less effective than others did. Lambda-cyhalothrin efficacy was significantly lower than spinosad at rate of 72 g a.i./ha (Bažok, 2016) [13].

Table 1: Number and reduction % in *A. ipsilon* larval population post 3 days of exposure to Acetamiprid+Lambda cyhalothrin and Lambda-cyhalothrin baits in potato field in El-Giza and El- Behaira Governorates during season 2020.

Insecticides	Total number of larvae, damage or cutting leaves in potato field during season, 2020					
	El-Giza Ggovernorate			El- Behaira Governorate		
	Before treatment	After 3 days of treatment	Reduction%	Before treatment	After 3 days of treatment	Reduction%
Acetamiprid+Lambda cyhalothrin	157	10	86.79	146	6	88.27
Lambda cyhalathrin	198	15	84.37	122	6	85.96
Control	163	79		117	41	

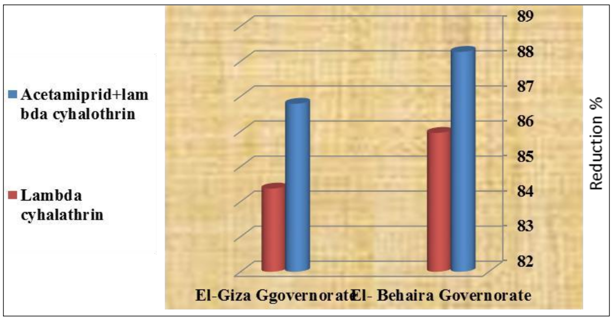

Fig 1: The reduction percentage in *A. ypsilon* larval population post 3 days of exposure to Acetamiprid + Lambda-cyhalothrin and Lambda-cyhalothrin baits in potato field in El-Giza and El-Behaira Governorates during season 2020.

It could be suggested that the application time of these pesticides is essential and the most suitable time for controlling *A. ipsilon* larvae is at once the appearance of the larval damage in the field, which led to keep the crop and increase the yield.

The effect of sub-lethal rate of the used insecticides on Total protein and total lipids activities in *A. ipsilon*

Sub-lethal rate of insecticides could be had a large stimulus on physiological and behavioral aspects as emergence%, adult fertility, sex ratio and all these affected by the changes in the enzyme secretions and other biological parameters. That could help in reducing the cost, application rate of insecticides and pollution of environment. Data presented in Table (2) and Fig (2), showed the result of sub-lethal field rate 125 and 100 ml of Acetamiprid+Lambda-cyhalothrin and Lambda-cyhalothrin as baits, on the major biochemical contents, total protein content and the total lipids in *A. ipsilon*, 4th instar larvae post 48 hrs of conduction. The effect was fluctuated in both protein and lipids between increase and decrease in the activities. Lambda-cyhalothrin showed highly significant increased to (9.81 g/dl) in the total protein in treated larvae while a remarkable significantly lower to (5.3 g/dl) with Acetamiprid + Lambda-cyhalothrin compared with untreated larvae (8.27 g/dl). The change in the activity was concluded that Acetamiprid+Lambda cyhalothrin caused highly significant inhibition in the protein synthesis (-35.91%) while, highly significant increasing on the total protein (18.65%) was generally exhibited by Lambda-cyhalothrin in treated larvae compared with untreated. Depending on the activity ratio, total protein was stimulated by (1.19) folds for Lambda-cyhalothrin and inhibited by (0.64) fold for Acetamiprid + Lambda cyhalothrin. Glycogen and proteins are the major biochemical component that necessary for chitin formation, insect reproduction and its development, where introduce in numerous reactions as lipids, hormone regulation, carbohydrates and proteins built-in the cell growth. The changes in the total protein content of the tested pests occurred by disruption in the hormonal system and the endocrine glands of insect which are responsible for the protein synthesis in insect body (Fahmy,2014)[19] Our results in harmony with Abd-El-Aziz *et al.*, (2020) [3] total protein of *S. littoralis* larvae was stimulated by treatment with Lambda-cyhalothrin + Thiamethoxam as (2.62) folds and as (1.4) folds with Lambda-cyhalothrin treatments. Abdou and Abdel-Hakim (2017) [6] protein content of total body of *A. ipsilon* larvae was generally exhibited with all tested methoprene concentrations except lower concentration slightly decreased, while the change % were 29.83% and 1.22% at concentrations 50 ppm and 25 ppm, respectively. Similar findings were observed by Fahmy, (2014) [19] the changes in total protein content during treatment the 4th larvae instar of *A. ipsilon* with both compounds showed a significantly higher in treated larvae compared with the untreated with gradual increase in total protein levels by time. Some reports observed the changes in biochemical and physiological parameter caused by various insecticides and other insect species, increasing protein content was determined by (Ghoneim *et al.,* 2012 and Basiouny *et al.,* 2016) [20-12] on *S. gregaria* and *S. littoralis* Also, Sharaby and EL-Dosary (2016) [36] reduction in total protein was in range 93.5 and 84.04%, where it was inhibited in the haemolymph and activated in gut protein, of the treated insect due to disturbance in the Juvenil and Ecdysteroid hormones. A significantly decrease in total proteins and carbohydrate that revealed to the antifeedant effect of the tested compounds, where the treated larvae disable to digest the diet (Bharat *et al.* 2017) [14].

Table 2: Effects of sub-lethal rates of Acetamiprid+Lambda cyhalothrin and Lambda-cyhalothrin on the total protein content and total lipids in the whole body extract of the 4th larval instars of *A. ipsilon* post-treatment for 3 days.

Tested insect		*A. ipsilon* larvae					
Enzyme activities		Total protein g/dl			Total lipids (ug/ml)		
Insecticides	Rates	Mean + SE	Change %	Activity ratio	Mean + SE	Change %	Activity ratio
Acetamiprid+Lambda cyhalothrin	125 ml	5.30+0.45^{c}	-35.91	0.64	171.7+2.01^{c}	-22.87	0.77
Lambda-cyhalothrin	100 ml	9.812+0.11^{a}	+18.65	1.19	312.5+3.67^{b}	+40.39	1.4
control	8.27+0.17^{b}				222.6+2.61^{a}		
F-value	0.0001***				0.000***		
L-S-D	0.969				9.67		

Total protein expressed as (g/dl) Total lipids expressed as (ug/ml) Means with the same letter are not significantly different at $p<0.05$
SE: Stander error.
Change %= test -control/control x100
Activity ratio: enzyme activity of tested compound / enzyme activity of control

The data in the same table revealed that a highly significant difference between insecticides and control. The treatment with Acetamiprid + Lambda cyhalothrin produced highly significant reduction in total lipids to 171.7 ug/ml, while Lambda-cyhalothrin caused highly significant increase to 312.5 ug/ml compare with 222.6 ug/ml in untreated control. The highest change % in total lipids + 40.39 with regard as a result of treated with Lambda-cyhalothrin, while Acetamiprid +Lambda-cyhalothrin caused highly significant inhibition -22.87% compare with untreated control**.** The total lipid activity was inhibited by (0.77) fold for Acetamiprid + Lambda cyhalothrin and stimulated by (1.4) folds for Lambda-cyhalothrin compare with untreated. The reduction in lipids may be due to the larvae use it as a of energy in case of starvation due to temporary stop feeding as a result of treatment that agree with Abd-El-Aziz, *et al.*, (2017) [2] the inhibitory effect on total lipids explained by the appearance of lack fat cell. Our results agree with Abdel-Hakim *et al.,* (2016) [4] the lipid content was reduced in *A. ipsilon* at high concentration and related to untreated larvae at concentrations 50 and 25 ppm. Many worker reports on other pests Rashwan, (2013) [33], significant reduction in *S. littoralis* lipids by -46.86% with coragen treatment. While, non-significant activity in the lipid post treatment with lufenuron at the lowest concentration of Sunn pest (Amiri *et al.*, 2010) [8]. Abd-El-Aziz *et al.*, (2020) [3] on *S. littoralis* total lipids had different effects, a significant stimulation by +4.87 with neonictinoid and inhibition by -6.71% with pyrethroid. The disturbance in the carbohydrates hydrolyzing enzymes inhibit all the functions during metamorphosis processes by reducing lipids, chitinase and protease (Abd-El-Aziz *et al.*, 2017) [2].

Enzymatic changes and the effect of sub-lethal rate of the used insecticides on acetyl cholinesterase activity in A**. *ipsilon*** larvae

An increasing on Acetyl cholinesterase was exhibited generally highly significance in *A. ipsilon* larvae treated with the sub-lethal field rate 100 and 125 ml /25 kg baits of both tested insecticides was summarized in Table (3) and Fig (2). The data cleared that highly significant increase in Acetyl cholinesterase was observed post treatment with Acetamiprid + Lambda-cyhalothrin (2428±10.8 µg Acetyl-cholinebromide/min/ml), in addition to (1657±56.59 µg Acetyl-cholinebromide /min/ml) in the larvae treated with Lambda-cyhalothrin in comparison with (429.3±5.04 µg acetyl-cholinebromide /min/ml) of untreated control. The highest significant induction percentage (+465.57%) noticed in Acetamiprid+ Lambda-cyhalothrin treatment followed by +285.98% in case of Lambda-cyhalothrin compared with control. The activity ratios in AchE recorded 5.66 and 3.86 folds with Acetamiprid + Lambda-cyhalothrin and Lambda-cyhalothrin, respectively as compare with control. Our results are in agreement with Abd-El-Aziz *et al.,* (2020) [2] revealed that the treatment of with Lambda-cyhalothrin + Thiamethoxam was the most stimulated AchE in *S. littoralis* larvae by (3.72) folds and by (2.54) folds with Lambda-cyhalothrin. This activity may be due to the hydrolysis as the mechanism of detoxification. In addition, moderate significant stimulation in AchE activity of 5th larval instars of *S. littoralis* to reached 25.74%. *A. ipsilon* caused by coragen, larvae were paralyzed quickly and stop feeding cessation shortly following ingestion Rashwan, (2013) [14]. Because of the neonicotinoids insecticide acting on the receptors of nicotinic acetylcholine in insect. In addition, Lambda-cyhalothrin is non–systemic pesticides which act on the insect nervous system by its disruption effect on sodium channel (MacBean, 2012) [29]. The results are in harmony with Nauen *et al.*,(2003,) by affecting on the insect muscle and causing paralysis, Ibrahim and Gabr (2020) [25] the effect of thiamethoxam on *A. ipsilon* larvae that didn't display quick knock down activity. Abd-El-Aziz *et al.*, (2017) [2] on *S. littoralis* AchE was significantly stimulated by 33.24% resulted from coragen treatment due to its mode of action. Where, Acetylcholine esterase hydrolyzes acetylcholine to avoid its build-up at nerve synapses, which led to disturbance in the nerve conduction with repeating firing. These disturbance in acetyl-cholinesterase may be synergistic and /or antagonistic, revealed to its immune system which strongly affect the active stress reaction that overreaction often as a results of *A. ipsilon* infection and leads to insects death (Muhammad Shakur, *et al.*, 2007 and Shairra *et al.*, 2016) [30, 35].

Enzymatic changes and the effect of sub-lethal rate of the used insecticides on Amylase activity in *A. ipsilon* larvae

Data tabulated in (Table, 3 and Fig. 2) revealed that the activity of the digestive enzyme, amylase in the treated *A. ipsilon* larvae with the sub-lethal field rate 125 and 100 ml/25kg baits of Acetamiprid+Lambda-cyhalothrin and Lambda-cyhalathrin.Non-significant differences in amylase activities that observed very closely to the control. Slightly increase in amylase (116±7.32 ug glucose/ min / ml) in case of Acetamiprid+Lambda-cyhalothrin treatment, while slightly decreased for Lambda- cyhalothrin treatment (99.97±6.31 ug glucose/min/ml) compared with (105.6±6.69 ug glucose/min/ ml) in control. The change %

showed non-significant increase (+9.85%) by Acetamiprid+Lambda-cyhalothrin, in addition, non-significant decrease (-5.05) in Lambda-cyhalothrin treatment in comparison of control. The activity ratio of amylase noticed no significant difference between two tested insecticides by 1.1 and 0.95 folds for Acetamiprid+Lambda cyhalothrin and Lambda-cyhalothrin, respectively.

Table 3 : Effects of sub-lethal rates of Acetamiprid + Lambda cyhalothrin and Lambda-cyhalothrin on Acetyl cholinestrase and Amylase activities in the whole body extract of the 4th larval instars of *A. ipsilon* post-treatment for 3 days.

Tested insect		*A. ipsilon* larvae					
Enzyme activities		AChE			Amylase		
Insecticides	Rates	Mean + SE	Change %	Activity ratio	Mean + SE	Change %	Activity ratio
Acetamiprid+Lambda cyhalothrin	125 ml	2428+10.8^{b}	+465.57	5.66	116+7.32	+9.85	1.1
Lambda-cyhalothrin	100 ml	1657+56.59^{a}	+285.98	3.86	99.97+6.31	-5.05	0.95
control	429.3+5.04^{c}				105.6+6.69		
F-value	0.000***				0.2986^{ns}		
L-S-D	239.24				23.059		

AChE expressed as µg Acetyl-cholinebromide/min/ml Amylase expressed as ug glucose/min/ml
Means with the same letter are not significantly different at $p<0.05$ SE: Stander error.
Change %= test -control/control x100 Activity ratio: enzyme activity of tested compound / enzyme activity of control

These results agree with that obtained by Abd-El-Aziz *et al.*, (2020) [1] the authors' stated that a significant inhibitory effect on amylase synthesis in *S. littoralis* as a result of treatment with Lambda-cyhalothrin + Thiamethoxam was significantly higher -32.47 than Lambda-cyhalothrin -20.61%, in addition to the change in invertase activity, significantly lower by -7.39% for Lambda-cyhalothrin+ Thiamethoxam while significantly higher +12.31% for Lambda-cyhalothrin. Also, significant decrease in amylase and invertase activities determined on *S. littoralis* larvae treated with abamectin (Abo El Ghar, *et al.*, 1995) [7].

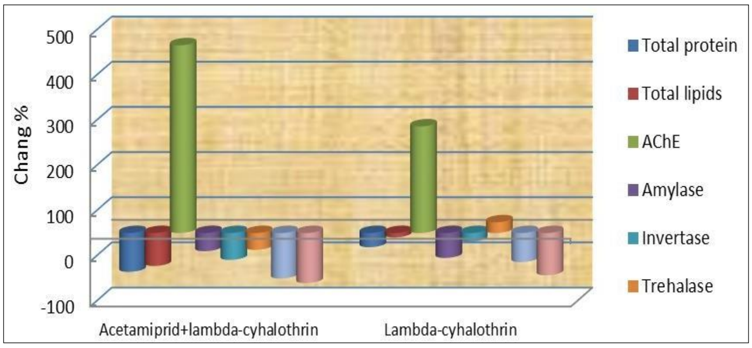

Fig 2: Changes percentage in the enzyme activities of the 4th instar larvae of *A. ipsilon* post-treatment with sub-lethal rate of Acetamiprid + Lambda-cyhalothrin and Lambda-cyhalothrin

Enzymatic changes and the effect of sublethal rate of the used insecticides on Ivertase and Trehalase in *A. ipsilon* larvae

Invertase and trehalase activities conducted in Table (4) and Fig (2). Invertase was significantly reduced from 49.51+3.12 ug glucose/min/ml for control larvae to reach 44.85+2.83 ug glucose/ min/ml and increased to 64.84+2.77 ug glucose/min/ml for treated larvae with Acetamiprid + Lambda-cyhalothrin and Lambda-cyhalothrin, respectively. The change % of invertase activity was significant increase +30.96% for Lambda-cyhalothrin treatment and non-significant decrease -9.41% for Acetamiprid + Lambda-cyhalothrin related to control. The activity ratio was 0.91 and 1.31 folds for Acetamiprid+Lambda-cyhalothrin and Lambda-cyhalothrin, respectively. The disturbance in the digestive enzyme and lipids interruption in food consumption as inhibition of digestion and absorption in the larvae, led to stop feeding, development and finally death. Fluctuation in the invertase activity was observed where activated (1.12) folds by Lambda-cyhalothrin and reduced by (0.93) fold in case of Lambda-cyhalothrin + Thiamethoxam treatment in the larvae of *S. littoralis* (Abd-El-Aziz *et al.*, 2020) [3].

The treatment with both insecticides causes highly significant increase in trehalase activity in treated larvae than control was reported in (Table 4 and Fig 2). Where the most stimulation 46.84+1.29 ug glucose/min/ml was in Lambda-cyhalothrin treatment, in addition was 30.39+0.91 ug glucose/min/ml in Acetamiprid + Lambda-cyhalothrin treatment compare with 26.96+0.93 ug glucose/min/ml in control. The change in trehalase activity was highly significant increase by +73.74 in case of Lambda-cyhalothrin followed by +12.72% for Acetamiprid+Lambda cyhalothrin. The activity ratio of trehalase activity recorded highly significant simulation by 1.74 and 1.13 folds for Lambda-cyhalothrin and Acetamiprid+ Lambda-cyhalothrin, respectively. Those agree with Abd-El-Aziz *et al.*, (2020) [3] Lambda-cyhalothrin+ Thiamethoxam revealed a highly significant increase in trehalase +67.56% and Lambda-cyhalothrin activated by + 32.36% in *S. littoralis* larvae.

Table 4: Effects of sub-lethal rates of Acetamiprid+Lambda-cyhalothrin and Lambda-cyhalothrin on Invertase and Trehalase in the whole body extract of the 4th larval instars of *A. ipsilon* post-treatment for 3 days.

Tested insect		*A. ipsilon* larvae					
Enzyme activities		Invertase			Trehalase		
Insecticides	Rates	Mean + SE	Change %	Activity ratio	Mean + SE	Change %	Activity ratio
Acetamiprid+Lambda cyhalothrin	125 ml	44.85+2.83^b	-9.41	0.91	30.39+0.91^a	+12.72	1.13
Lambda-cyhalothrin	100 ml	64.84+2.77^a	+30.96	1.31	46.84+1.29^c	+73.74	1.74
control	49.51+3.12^b				26.96+0.93^b		
F-value	0.0061**				0.000***		
L-S-D	9.886				3.604		

Invertase and Trehalase expressed as ug glucose/min/ml.
Means with the same letter are not significantly different at $p<0.05$ SE: Stander error.
Change %= test -control/control x100 Activity ratio: enzyme activity of tested compound / enzyme activity of control

Insect carbohydrate reserves are existing as glycogen and trehalose, which can be speedily converted to glucose for chitin synthesis (Chippendale, 1978) [16]. Glycogen converted to glucose in control insects, which was integrated in new cuticle synthesis. However, glycogen levels remained high, in case of treated insects that due to the inhibition of chitin synthesis. Fahmy (2014) [19] the increase could explain via the natural increase of detoxifying and protective hydrolytic enzymes that usually occur post treatment. In contrast, the inhibition of total protein synthesis post three days of treatment considered as a first sign of cell death. In addition, the inhibitory effect on the total lipids and carbohydrate, of *S. littoralis* larvae may be due to a chain effect starting mainly from inhibition of chitin synthesis. The disturbance in the digestive enzyme and lipids interruption in food consumption as inhibition of digestion and absorption in the larvae which led to stop feeding, development and finally death (Rashwan, 2013, Abd-El-Aziz *et al.,* 2017 and 2020) [33, 2, 3].

Enzymatic changes and the effect of sublethal rate of the used insecticides on Acid and Alkaline phosphatase in *A. ipsilon* larvae

The obtained data in Table (5) and Fig (2) showed highly significant effect in both acid and alkaline phosphatase activity because of treatment with both tested sub-lethal rates of insecticides. The activity of acid phosphatases of *A. ipsilon* elevated a highly significant decrease to reach 4.09+0.05 and 7.09 +0.57 ug/min/ml with the treatments of Acetamiprid+Lambda-cyhalothrin and Lambda-cyhalothrin, respectively related to 8.28+ 0.31 ug/min/ml of controls. The changes percentage in the acid phosphatase activity reflected a significant inhibition as a result of treatment with Acetamiprid + Lambda-cyhalothrin and Lambda-cyhalothrin by -50.60 and -14.37% respectively. The activity ratio of acid phosphatase most decline to be 0.49 fold in Acetamiprid + Lambda-cyhalothrin and 0.86 fold in Lambda-cyhalothrin samples as comparison with control.

Table 5: Effects of sub-lethal rates of Acetamiprid+lambda cyhalothrin and Lambda-cyhalothrin on Acid and Alkaline phosphatase activities in the whole body extract of the 4th larval instars of *A. ipsilon* post-treatment for 3 days.

Tested insect		*A. ipsilon* larvae					
Enzyme activities		Acid phosphatase			Alkaline phosphatase		
Insecticides	Rates	Mean + SE	Change %	Activity ratio	Mean + SE	Change %	Activity ratio
Acetamiprid+Lambda cyhalothrin	125 ml	4.09+0.05^b	-50.60	0.49	82.5+16.18^a	-60.87	0.39
Lambda-cyhalothrin	100 ml	7.09+0.57^a	-14.37	0.86	119.17+9.34^a	-43.48	0.57
control	8.28+ 0.31^a				210.83+9.34^b		
F-value	0.0005***				0.0007***		
L-S-D	1.28				40.951		

Acid phosphatase expressed as ug phosphate /min/ml. Alkaline phosphatase expressed as ug phosphate /min/l.
Means with the same letter are not significantly different at $p<0.05$ SE: Stander error.
Change %= test -control/control x100 Activity ratio**: enzyme activity of tested compound / enzyme activity of control

On the light of same trend, *A. ipsilon* alkaline phosphatase confirmed highly significant reduction in its activity to reach its maximum (82.5 ug/min/ml) in larvae treated with sub-lethal rates of Acetamiprid +Lambda-cyhalothrin while 119.17 ug/min/ml with Lambda-cyhalothrin compared with 210.83 ug/min/ml in control and non-significant difference between each other. The change percentage reflects reducing in the enzyme activity where the most reduction (- 60.87%) as a result of Acetamiprid+Lambda cyhalothrin treatment while Lambda-cyhalothrin reduced by -43.48% compared to control. Relatively to the activity ratio of enzyme was dropped to 0.39 and 0.57 fold than control for Acetamiprid + Lambda-cyhalothrin and Lambda-cyhalothrin, respectively. Both insecticides had the similar effect on AchE, trehalase, acid and alkaline phosphatase and different effect on total protein, total lipids, invertase and amylase production. That fact may be due to the two active ingredients, combination of acetamiprid and Lambda-cyhalothrin where Lambda-cyhalothrin its active ingredients belong to the group of pyrethroids (non-systemic) and neonicotinoids (acetamiprid). The reduction in both acid and alkaline phosphatase activities as response of treatments that related to the harmful of insecticide's effect on the digestive system of insect's and its development where alkaline phosphatase play an important role in insect physiology and digestive system that agree with (Bharat *et al*. 2017) [14] on *Helicoverpa armigera*. Abd-El-Aziz *et al.,* (2020) [3] the activity of alkaline phosphatase on *S. littoralis* was fluctuated with the effect of tested insecticides where reduced to (0.74) fold for Lambda-cyhalothrin +Thiamethoxam treatment while activated by (1.42) folds for Lambda-cyhalothrin. On the other hand, acid phosphatase was activated by (1.0) fold in Lambda-cyhalothrin + Thiamethoxam and (1.38) folds in Lambda-cyhalothrin treatments. Also, the using of sub-lethal rates of certain neonicotinoids and pyrethroids was studied on different physiological and histological aspects considered excellent controlling option in IPM system for both *A. ipsilon* and *S. littoralis* (Abd-El-Aziz *et al.*, 2019, 2020 and Joshi, *et al.*, 2020) [1, 3, 28]. From the previous results, Acetamiprid+Lambda-cyhalothrin caused highly significant inhibition, in total protein, total lipids, invertase, acid and alkaline phosphatase synthesis. While stimulate significantly AchE, trehalase and non-significant in amylase synthesis of treated larvae.

Lambda-cyhalothrin caused significant inhibition in acid, alkaline phosphatase and non-significant in amylase production. On contrast a highly significant stimulation in AchE and total protein, total lipids, invertase and trehalase synthesis was observed. We suggested that the application time of these pesticides is essential and the most suitable time for controlling *A. ipsilon* larvae is at once the appearance of the damage in the field in the early grown plant by larvae. In addition, the wise apply of sub-lethal field rate with various degrees of success can suppress pest populations, avoid buildup the resistance, led to keep the crop and increase the yield.

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