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RESEARCH ARTICLE

IMPACT OF CERTAIN INSECT GROWTH REGULATORSON SPODOPTERA LITTORALIS (LEPIDOPTERA: NOCTUIDAE)

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Abstract

This study aims to evaluate the toxic and biochemical effect of certain insect growth regulators (IGRs) namely, lufenuron and hexaflumuron on S. littoralis. Our results indicated that lufenuron was more toxic than hexaflumuron on 4th instar based on LC_{50} values. In addition, chitinase activity was increased of treated insects during moulting period than control while the chitin content of treated larvae was lower than control during this period. Glycogen level of treated larvae increased than control ones.Moreover, total protein, total lipids and total carbohydrates were significant decreased for lufenuron at 12 hrs post treatments compared with hexaflumuron. In addition to amylase and trehalase were significant decreased in case of hexaflumuron compared with lufenuron. On contrast, both insecticides caused non significant inhibiti on in lipase and invertase compared with control. Results indicated that lufenuron and hexaflumuron have shown high potentiality against S. littoralis.

Introduction:-

The cotton leafworm S. littoralis (Boisd.) is considered as a destructive phytophagous pest causing great losses in yield (Hamouda and Dahi, 2008, Hatem et al., 2009; Lanzoni et al., 2012). Major of conventional chemical insecticides used against S. littoralis led to resistance development. Hence, it is important to search for alternative and eco-friendly methods of pest control. IGRs interfere with insect development and growth. Chitin synthesis inhibitors (CSIs) are among IGRs which prevent insect ecdysis via interference with biosynthesis of chitin and complete life cycle (Hammock &Quistad, 1981; Mondal &Parween, 2000). Many insect pests as well as different lepidopterous insects were controlled by using IGRs (Abd-El Wahed et al., 2011; Assar et al., 2016). Therefore, the present study was conducted to evaluate the toxic and biochemical effects of certain chitin synthesis inhibitors namely lufenuron and hexaflumuron on 4th instar of S. littoralis.

Materials and Methods:-

Insects:-

From the cotton leafworm division, Plant Protection Research Institute, Dokki, Egypt,S. littoralis larvae were obtained. Larvae were reared on castor bean leaves at 27±2° C and 65±5 R.H. as described by (El-Dafrawi et al., 1964).

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Experiments:-

Toxicity bioassay:-

Lufenuron (Match® 5% EC) and hexaflumuron (Consult® 10% EC) were supplied by Syngenta. Leaf dipping method was used for experiment. Castor leaves were dipped in six concentrations (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 ppm) and (0, 20, 40, 60, 80, 100, 120 ppm; prepared in water) of lufenuron and hexaflumuron, respectively for 30 seconds. Also, for control ones untreated leaves were put in tap water and allowed to fed 4th larval instar on them. Each treatment contains 3 replicates and each replicate has 10 larvae of the same age. After 48 h, the mortality was recorded and mortality % was corrected according to Abbott's formula (Abbott, 1925) and LC_{50} values calculation was determined by Probit analysis and related parameters, according to Finney (1971) using software computer program (SAS, 2002).

Chitinase assay:

The method of Ishaaya&Casida (1974) was adopted for chitinase activity estimation.

Chitin determination:-

The chitin was extracted from S. littoralis by the method of Kaya et al. (2017).

Glycogen determination:-

The method of Yuval et al. (1998) was adopted for determination of glycogen level.

Total carbohydrates level estimation:-

The method of Yuval et al. (1998)was adopted for the carbohydrate level estimation.

Total protein level estimation:-

The method of Bradford (1976) was adopted for total protein measure.

Total lipid level estimation:-

The method of Yuval et al. (1998) was adopted for lipid content measure.

Protease assay:

The method of Birk e al. (1962) was adopted for proteolytic activity measure.

Lipase assay:

The method of Tahoun& Abdel-Ghffar (1986) was adopted for lipase activity measure.

Enzymes hydrolyzing carbohydrate assay:

The method of Ishaaya&Swiriski (1976) was adopted for estimation of amylase, invertase and trehalase measure.

Statistical analyses:-

Means ± SEs of the experimental values in tables were obtained and data were subjected to student's t-test, to evaluate the significance of the results at levels of 1, 5.

Results and Discussion:-

Table (1) showed that LC_{50} values of lufenuron and hexaflumuron against S. littoralis 4th larval instar were 0.304 and 59.223 ppm respectively with a slope value of 3.884±0.294 and 4.1055±0.3042 for 4th instar larvae, respectively. The LC_{50} of lufenuron to S. littoralis 4th instar was found to be 0.0188 ppm (El-Banna, 2020). While the values of LC_{50} were 1.3, 1.66, 1.7 and 0.81 ppm for hexaflumeron, teflubenzuron, diflubenzuron and lufenuron, respectively against Pectinophoragossypiella 3rd instar (Taha & Radwan, 2023).

Table 1: Lethal action (as expressed by LC_{50} values) of lufenuron and hexaflumuron on *S. littoralis* 4th larval instar.

Treatments	LC_{50} ppm*	95% fiducial limits		Slope	χ^2
		Lower	Upper		
Lufenuron	0.304	0.229	0.380	3.884±0.294	20.211
Hexaflumuron	59.223	46.452	71.873	4.106±0.304	17.079

* LC_{50} values are significant (P< 0.05), whenever fiducial limits do not overlap.

The results in table (2) showed that during intermoult period and post-treatment with 2,12 and 24 hr, the chitinase activity in larvae treated with lufenuron increased insignificantly than control ones by 0.457, 3.318 and 3.815 %, respectively, while in case of hexaflumuron, the activity insignificantly decreased by 2.739, 1.89 and 1.635%, respectively. Futhermore, during moulting, the activity of chitinase in larvae treated with lufenuron significantly increased than control ones by 17.764% while that of hexaflumuron increased significantly by 19.35%. El-Banna (2020) stated that chitinase values was increased in both chlorfluazuron and lufenuron being 266.3 and 230.3 (µg NAGA/min/g.b.wt), respectively against S. littoralis 4th larval instar compared with control. Our results agreed with those of Abdel Aziz (2019) and Abd El-Mageed&Shalaby (2011), they found when treated S. littoralis with mixtures of insecticides and IGRs, chitinase activity was increased. Abdel-Mageed et al. (2018) found that treatment of S. littoralis with chlorfluazuron, triflumuron and flufenoxuron, increase in chitinase activity by 51.28%, 72.06% and 32.70% for, respectively compared with control.

Chitinase enzyme is vital for ecdysis in insects. The insect cuticle might constitute a useful target site for different insecticides. As a result of treatments with different insecticides, it might affect synthesis of protein within insects leading to obtained change in this enzyme activity (Dean et al., 1999; Merzendorfer&Zimoch, 2003; Kostyukovsky&Trostanetsky, 2006).Data presented in table (3) showed that chitin content in both treatments was significantly decreased than control during moulting period being 1.064, 1.191 and 1.580 mg, respectively. Chitin synthesis inhibition impedes chitin biosynthesis in treated insects. Malfunction in chitin biosynthesis prevents molting and or leading to defective insect cuticle (Hammock &Quistad, 1981).

Table 2: Chitinase activity in the total body homogenate of S. littoralis treated with lufenuron- and hexaflumuron-LC_{50}s.

Hours after treatment	Control	Lufenuron		Hexaflumuron	
		Activity (a)	% (b)	Activity (a)	% (b)
2	**219±7.31**	**220±03.11** ns	+ 0.457	**213±01.02** ns	- 2.739
12	**211±1.95**	**218**±01.93 ns	+ 3.318	**207±05.15** ns	- 1.890
24	**367±1.10**	**381±06.2**0 ns	+ 3.815	**361**±12.**50** ns	- 1.635
48	**5421±32.13**	**6384±70.6**0 ***	+ 17.764	**6470**±08.**41*****	+19.350

(a) Activity expressed as as µg NAGA released x10^3 /min/insect .
(b) Percentage decrease or increase than untreated .
Ns Non-significant .
*** Significant at the level of 0.1%

Table 3: Chitin content of S. littoralis treated with lufenuron- and hexaflumuron-LC_{50}s.

Hours after treatment	Chitin content (mg)		
	Control	Lufenuron	Hexaflumuron
2	1.50±0.05	1.172±0.06	1.072±0.01
12	2.165±0.10	1.251±0.04	1.411±0.04
24	3.10±0.06	1.647±0.06	1.748±0.03
48	1.580±0.13	1.064±0.03***	1.191±0.02 ***

Data are presented as means ± SE_S
*** Significant at the level of 0.1%
Concerning the increase in glycogen level of treated larvae compared with control as indicated in table (4) it can be referred to that chitin synthesis in treated larvae was lower than control ones, hence requirement of glycogen for chitin synthesis was lesser. These results were agreed with that of Radwan et al. (1986).

Table 4: Glycogen level during moulting of S. littoralis treated with lufenuron- and hexaflumuron- LC_{50}s.

Treatments	Fresh body weight (mg)	Glycogen level	
		as µg glucose/insect	% of body weight
Control	41±1.46	104.81±2.90	0.26
Lufenuron	37.5±2.00	127.4±2.45	0.33***
Hexaflumuron	40.83±0.80	142.28±4.00	0.34***

Data are presented as means ± SE_S
*** Significant at the level of 0.1%

The results indicated that total protein in larvae treated with lufenuron at 12 and 24 hr post treatment were significantly decreased than control being 1251 and 1171.53 µg/ larva, respectively compared with that of control (1348.2 and 1623.1 µg/ larva, respectively) (Table 5). While in case of hexaflumuron was non-significantly increased than control at 12 hr post-treatment (1358.02 µg/ larva), but at 24 hr post-treatment it significantly decreased (947 µg/ larva). Similar results were obtained by El-Banna (2020) who found that S. littoralis protein content was decreased after IGRs treatment compared with control ones. The heamolymph protein works as a reserve source of protein synthesis responsible for development and growth of the adult during pupal stage (Florkin&Jeanulaux, 1964). Also, total lipids in both treatments with lufenuron and hexaflumuron either at 12 or 24 hr post treatment were significantly increased than that of control. While Assar et al. (2016) showed that S. littoralis treated with teflubenzuron and hexaflumuron, respectively led to total lipid reduction. Concerning total carbohydrates, the results indicated that both lufenuron and hexaflumuron either at 12 or 24 hr post treatment were significantly increased than control. While novaluron increased the total carbohydrates in S. littoralis 5th larval instar (Aly & Ali, 2024). The efficacy of the IGRs relies on the species of treated insect and the applied concentration (Khedr et al., 2005).

Table 5: Effects on the main metabolites of S. littoralis treated with lufenuron- and hexaflumuron-LC_{50}s.

Treatments	Total carbohydrates (µg glucose/larva)		Total lipids (mg/larva)		Total protein (µg/larva)	
	12 hrs ●	24 hrs ●	12 hrs ●	24 hrs ●	12 hrs ●	24 hrs ●
Control	194.1±2.9	243.0±1.8	6.9±0.10	8.42±0.55	1348.2±7.5	1623.1±9
Lufenuron	250±1.9***	269.88±1.55***	7.95±0.195***	9.37±0.1*	1251±2.8***	1171.53±6.8***
Hexaflumuron	262.70±0.9***	301.84±6.6***	8.24±0.11***	9.17±0.25*	1358.02±5.4 n.s.	947.0±5.7***

Data are presented as means ± SE_S
● hours post treatment
n.s. Non-significant
* Significant at the level of 5%
*** Significant at the level of 0.1%

Table (6) shows that protease activity significantly increased in both lufenuron and hexaflumuron compared with that of control. While lipase activity was insignificantly affected by treatment. In case of carbohydrates hydrolyzing enzymes, the amylase activity was significantly decreased in both lufenuron and hexaflumuron, Trehalase activity was insignificantly decreased in lufenuron but significantly decreased in case of hexaflumuron. While invertase activity was insignificantly affected by treatments. Ishaaya& Ascher (1977) indicated that carbohydrates might be affected due to the reduced level of amylase, invertase and trehalase of Triboliumcastaneum 4th instar larvae treated with diflubenzuron. Also, El Saidy&Degheele (1990) agreed with our results in that amylase activity was reduced in S. littoralis after diflubenzuron treatment. Thus, our results showed that chitin synthesis inhibitors do not reduce digestive enzymes activity, except some carbohydrates hydrolyzing enzymes.

Table 6: LC_{50} effects of lufenuron and hexaflumuron on S. littoralis digestive enzymes.

Treatments	Digestive enzymes				
	Amylase (µg glucose/min/gut)	Trehalase (µg glucose/min/gut)	Invertase (µg glucose/min/gut)	Lipase (µM oleic acid liberated/min/gut)	Protease (O.D. units x 10^3 / min./gut)
Control	1.601±0.07	21.17±0.74	51.182±0.85	1.21±0.12	2.70±0.18
Lufenuron	1.234±0.11**	20.752±1.3n.s.	50.01±0.65 n.s.	1.24±0.11 n.s.	4.33±0.15***
Hexaflumuron	1.031±0.07***	11.251±0.62***	51.07±0.45 n.s.	1.20±0.14 n.s.	3.52±0.10**

Data are presented as means ± SE_S
n.s. : Non-significant
** : Significant at the level of 1%
*** : Significant at the level of 0.1%

Conclusion:-

The tested IGRs effectiveness was shown from the latent effect on some biochemical aspects. Therefore, these CSIs will disrupt many physiological functions and ultimately lead to death. In addition, these results would facilitate better integration of these insect growth regulators into integrated management programs of S. littoralis.

Competing interests:
No competing of interests was obtained.

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