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The effect of some insecticide and insect growth regulator on cotton leaf worm *Spodoptera littoralis* (Lepidoptera, Noctuidae) under laboratory condition

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Abstract:

The effect of amidochlorochloride, lambda and biflorate (IGR) against 2nd and 4th instars of the laboratory strain of *Spodoptera littoralis* (Boisduval). (Lepidoptera: Noctuidae) was calculated under laboratory conditions. Amidochlorochloride was the most toxic compound against the 2nd and 4th larval instars of *S. littoralis*. All the treated larvae were biologically affected by the three tested compounds. The result was varied according to the larval instars and tested compounds. Therefore, the treated larvae were resulted in decreased pupation and adult emergence percentages, even so it reaches to 0% in the 2nd instar treated with amidochlorochloride had the strongest effect in this respect. The treatment of 2nd instar with the three compounds induced the highest increase in larval mortality, least pupation percentage and also least adult emergence percentages. Hence, the larval treatment of 2nd and 4th instars with amidochlorochloride, lambida and biflorate gave the shortest period of adult longevity, as compared to control. The larval treatment of 2nd and 4th instars with the three tested compounds increased the adult emergence, the treatment of 2nd and 4th instars with amidochlorochloride, lambida and biflorate (IGR) had the strongest effect in this respect than control.

Introduction

The cotton leaf worm *Spodoptera littoralis* (Boisduval). (Lepidoptera: Noctuidae) considered as one of the most serious and destructive pests not only for cotton plant, but also for other vegetable and field crops in Egypt. This insect has a complete developmental stage this mean that has four stages (Adults, egg, larval stage and pupal stage), after coupling male with the female, the female moth lays prominent masses of egg (20 to 1000 eggs) on the underside of the cotton leaf which far about 50 cm above the ground (El-Saadany and Abdel Fattah, 1976).

Such larval stage is the longest and most destructive

stage in terms of physical damage to plant tissues. As the insect develops, it completes six instars. The first three instars feed mainly on the lower surface of the cotton leaves, whereas later instars feed on both surfaces. The third and fourth instars remain on a plant but do not feed during daylight.

Later instars migrate off the plant and rest in the soil during the day and return to the plant at night. When the temperature increases, the young larvae hide under the leaves and the older ones move to the soil (Gawaad and El-Gayar, 1974 and Abdel-Megeed and Iss Hak, 1975).

In the pupal stage the insects remain at 3-5 cm depth below the soil surface. Pupa creates "a clay cell" or cocoon in which it usually pupates within five to six hours. Emergence of moths occurs at night, and they have a lifespan of five to ten days. Adults fly at night, mostly between the hours of 8 pm and midnight. About half of females will lay their eggs before sunrise of the same night of mating. The adult moth is very active, agile and becomes capable of longer flights (Salama and Shoukry, 1972).

The aim of the present work is to study the effect of amidochlorochloride, lambda and biflorate (IGR) against the 2nd and 4th instars of the laboratory strain of *S. littoralis* was calculated under laboratory conditions.

Materials and methods

1. Maintenance of insect culture:

The culture of *S. littoralis* is used in this study originated from eggs obtained by a susceptible strain established in Cotton Leaf Worm Department, Plant Protection Research Institute, Agricultural Research Center , Giza, Egypt. This strain was reared in the laboratory under constant laboratory conditions of 25 ± 2 ºC and 65 ± 5 % R.H. according to El-Defrawi *et al*. (1964).

Egg masses were kept in petri dishes, 2 egg masses in each dish, until hatching. The hatched larvae were transferred to glass jars (2 liter capacity) covered with muslin cloth secured with rubber bands larvae were provided daily with fresh Castor leaves (*Ricinus communis*).

The larvae develop reaching for the 6th instar, pre-pupae were allowed to pupate in glass jars containing 2 inch layer of dry saw dust, the resulting pupae were then placed on filter paper discs in uncovered petri- dishes, which were kept I 1 cubic foot wire screen cages.

The resulting moths were fed on 20% sugar solution and allowed to lay their eggs on fresh *Nerium oleander* leaves as a physical surface for moths mating, ovipositor and resting egg- masses were collected after 2 days and transferred to petri- dishes for another generation.

The effect of these treatments was studied on 2nd and 4th instar larvae of cotton leave worm.

2. The tested compound:

2.1. Amidochlorochloride 35%

2.2. Lambda:

CASName: (R)-cyano(3-phenoxyphenyl)methyl(1S,3S)-rel-3-[(1Z)-2-chloro-3,3,3-trifluoro-1-propenyl]-2,2-dimeth

Molecular
Formula: C23H19ClF3NO3

2.3. Biflorate (IGR) 25% WB

By a concentration 0.25, 0.50 and 1.00% for each treatment three replicate for these treatments each have 20 larvae in addition to untreated check control (Treated with fresh water).

3. The following procedures were applied:

3.1. For each concentration of any tested treatment, three replicates, of 20 2nd and 4th instar larvae, placed in a jar for rearing to feed on the caster bean leaves treated with the different concentrations of Treatments.

3.2. Mortality rates were recorded every 2 days. Larvae that survived after treatment were transferred to other jars containing untreated caster bean leaves.

3.3. Before exposing the larvae to treated food, they were starved for 4 hours in order to obtain rapid simultaneous ingestion of the contaminated food.

3.4. Control test was conducted by dipping clean caster bean leaves in water, left to dry and then offered to the experimental larvae.

3.5. The experiments were carried out under laboratory conditions of 25 ± 2°C and 65 ± 5 % RH.

3.6. The castor-bean leaves were dipped for one minute in each of the used concentrations, and then treated leaves were left for air dryness and offered to the tested larvae.

4. Studying effects on different developmental stages:

For studying the latent effect, other samples were taken each 2 days and introduced to the rest a live larva until pupation stage. Mortality count was recorded each 2 days then mortality percentages were calculated, developmental effect against both pupae and moth emergency was studied by recording total numbers of formed pupae and moth emergency for each treatment then calculating their percentages by method publishing by El-Sisi and Farrag (1989) as the following:

$$\textbf{Percentage of Pupation} = \frac{\text{No. of formed pupae}}{\text{Initial No. of 2nd or 4th instar larvae}} \times 100$$

$$\textbf{Percentage of moth emergency} = \frac{\text{No. of formed moth}}{\text{Initial No. of 2nd or 4th instar larvae}} \times 100$$

Antifeedent, effect of different treatments against 2nd and 4th instars larvae was determined by introducing accurately the weight of the bean leaves treated with concentration mentioned before compared with un-treated which fed with un-treated bean leaves. After 48 hrs. of feeding, the rest leaves were weighted in each replicate, then consumed amount of leaves were calculated and antifeedent effect were calculated as Waldbauer (1968) equation:

$$\textbf{Percentage of Antifeedent} = \frac{Cc - Ct}{Cc} \times 100$$

Where: Cc = Consumped amount in un-treated.
Ct = Consumped amount in treated.

Results and discussion

Data in Table (1) showed the effect of three compounds(Two insecticides, amidochlorochloride, lambida and biflorate (IGR) with serial concentrations (0.25, 0.50 and 1 %) on the 2nd instar larvae of cotton leafworm under laboratory conditions. In addition to the developmental effect in pupation and moth emergency.

The obtained results showed for all compounds used that there was a regular direct relationship for each concentration between the percentage

of mortality and the increase in the period of treatment. Amidochlorochloride on using 0.25 % showed 25.00, 45.00, 62.00, 86.00 and 90.00 % mortality after 2, 4, 6, 8 and 10 days from treatment, respectively, and on using 1 % of amidochlorochloride, the mortality percentage changed from 48.00, 64.00, 90, 99.00 and 100 % after the same periods of treatment.

Furthermore for all tested concentrations for each compound after the same period of treatment, there was also a regular direct relationship between the increase in concentration and the percentage of mortality, lambida after 2 days from treatment, the mortality percentage changed from 15.00, 24.0, to 40.00 % and after 4 days from treatment, it changed from 39.00, 57.00 to 65.00 % and after 8 days, it changed from 85.00, 92.00 to 95.00 % for the three concentrations used respectively. For the developmental effect, the three compounds showed fluctuations in both percentage of pupation and the percentage of moth emergency, the variation was not only between the three compounds but also between the used concentrations of the same compound, the percentage of pupation in the case of biflorate changed from 55.00 to 60.00 and returned back to 58.00 %. The same result was noticed with the percentage of moth emergency for the same compound as it changed from 54.00 to 59.00 and returned back to 55.00 %. The direct proportionation between concentration and percentage of mortality agreed with El-Khayat *et al.* (2012), reported that the second instar larvae reflected higher level of susceptibility towards all the tested insecticides that included Insect growth regulators (Nomolt 15% Mimic 24% an Runner 24%); Bio-insecticides, Tracer , XDE and Dipel 2x ;and Organophosphorus (Chlorpyrifos) than fourth one.

Also, Haggag (2013) found that Bt-formulations named Dipel DF, Dipel 2X and Delfin tested against 2nd and 4th instars larvae of *S. littoralis* were highly killed at the initial time, followed by agry, protecto and agerin formulations, respectively, who reported that, the increase either in the concentration of insecticides or the period of treatment resulted in an increase in the percentage of mortality. While the fluctuations in the percentage of pupation and the percentage of moth emergency may be explained on the basis of population individuals tolerance, any population consisting of individuals varies intolerance, sensitive individuals will die directly while tolerant individuals will remain whatever the concentration used or the period of treatment (Mohamed and El-Kady, 2010).

Table (1): Effect of amidochlorochloride, lambida and biflorate on 2nd instar larvae of *Spodoptera littoralis* under laboratory conditions.

Compounds	Conce-ntration (%)	Mortality % after days					Developmental effect	
		2	4	6	8	10	% pupation	% moth emergency
Amidochlorochloride	0.25	25.0	45.0	62.0	86.0	90.0	5.0	1.00
	0.50	30.0	56.0	88.0	95.0	100.0	00.0	00.0
	1.00	48.0	64.0	90.0	99.0	100.0	00.0	00.0
Lambida	0.25	15.0	39.0	50.0	85.0	97.0	00.0	00.0
	0.50	24.0	57.0	89.0	92.0	100.0	00.0	00.0
	1.00	40.0	65.0	89.0	95.0	100.0	00.0	00.0
Biflorate	0.25	10.0	19.0	23.0	32.0	40.6	55.0	54.0
	0.50	18.0	24.0	29.0	37.0	39.0	60.0	59.0
	1.00	20.0	25.0	33.0	39.0	42.0	58.0	55.0

Also, Haggag (2013) found that Bt-formulations named Dipel DF, Dipel 2X and Delfin tested against 2nd and 4th instars larvae of *S. littoralis* were highly killed at the initial time, followed by agry, protecto and agerin formulations, respectively, who reported that, the increase either in the concentration of insecticides or the period of treatment resulted in an increase in the percentage of mortality. While the fluctuations in the percentage of pupation and the percentage of moth emergency may be explained on the basis of population individuals tolerance, any population consisting of individuals varies intolerance, sensitive individuals will die directly while tolerant individuals will remain whatever the concentration used or the period of treatment (Mohamed and El-Kady, 2010).

The data showed the effect of the three previously mentioned compounds with the same concentrations on the 4thinstar larvae under laboratory conditions, relatively the results found were as in the case of the 2nd instar larvae, direct proportionation between the percentage of mortality and the period of treatment for each concentration, in addition to the increase in the percentage of mortality with the increase in concentration for the same period of treatment. Furthermore, the developmental effect for pupation and moth emergency showed the same fluctuations as in the case of the 2nd instar larvae, the results that may be attributed also to population tolerance (Mohamed and El-Kady, 2010).

Data presented in Table (2) showed the antifeedant effect of the three used compounds with the same concentrations on both 2nd and 4thinstar larvae under laboratory conditions. For all used compounds and for both stages, an inverse proportionation was obtained between the increase in concentration and the antifeedant effect. Amidochlorochloride showed 90.00, 70.00 and 60.00 % antifeedant effect on using 0.25, 0.50 and 1.00 % respectively.

Table (2): Antifeedent effect of both 2nd and 4th instar larvae of *Spodoptera littoralis.*

Compounds	Concentration (%)	2nd instar larvae		4th instar larvae	
		Consumed %	Antifeedant %	Consumed %	Antifeedant %
Amidochlorochloride	**0.25**	3.0	97.0	10.0	90.0
	0.50	15.0	85.0	30.0	70.0
	1.00	25.0	75.0	40.0	60.0
Lambida	**0.25**	10.0	90.0	5.0	95.0
	0.50	18.0	82.0	15.0	85.0
	1.00	30.0	70.0	20.0	80.0
Biflorate	**0.25**	3.0	97.0	3.0	97.0
	0.50	5.0	95.0	5.0	95.0
	1.00	10.0	90.0	4.0	96.0
Control		95.6	4.4	100	0.0

The results could be explained on the basis of leaf composition and the increase in insecticide concentration, the leaf consists of wax and fat, organic materials that facilitate penetration of the insecticide, as its concentration increased inside the leaf. The softness of the leaf may be affected and as a result the insect may refuse to feed on it (Mesbah *et al*., 2000 and El-Naggar, 2009) indicated farming practices that cause nutrition in balance can lower pest resistance. Meyer (2000) proposed that soil nutrient availability not only affects the amount of damage that plants receive from herbivores but also the ability of plants to recover from herbivores. Ramesh *et al.* (2005)

conducted that organic crops have been shown to be more tolerant as well as resistant to insect attacks. Saad and Nabil (2012) were studied the effects of some foliar fertilizers on the biology of silkworm, *Bombyx mori* L. In this study, the authors investigated the possibilities of using four compounds as foliar fertilizers to determine the new beneficial effects on larval and pupal mortality and larval, pupal weights, finally, the effect of these compounds on some biological aspects of cotton leaf worm, *S. littoralis.*

1. Larval and pupal duration:

Data presented in Tables (3 and 4) indicated that the 2nd and 4th larval instars of *S. littoralis* fed on castor oil leaves treated with amidochlorochloride, lambida and biflorate compounds induced a highly significant ($p<0.01$) increase of the larval duration. The effect was more pronounced with the larval treatment of 2nd larval instar with the three tested compounds, it averaged 18.9+1.0, 17.8+ 3.1 and 18.0+ 2.5 days, respectively, as compared with 14.3+ 1.0 days of control. While the 4th instar larvae fed on lambida, gave the highest significant ($p<0.01$) increase in the larval duration to average 16.5+ 1.3 days, as compared to 14.3+ 1.3 days of control. Whereas, the treatment of 4th instar with both amidochlorochloride and biflorate compounds caused equal significant increase in the larval duration to average 12.8+1.1 and 14.9+1.4days, respectively, as compared to that of control (13.2days) (Table 5). Treatment of the 2nd and 4th instar larvae of *S. littoralis* with the three compounds showed highly significant ($p<0.01$) increase in the pupal duration.The effect was more noticeable with the treatment of 2nd instar with the three compounds of control .Whereas, the 4th instar treated with the three compounds gave significant ($p<0.01$) increase in the pupal duration.

Lambida treatment caused a higher prolongation to pupal duration averaged 19.0 days, as compared to 14.4 days of control. While, the larval treatment of 4th instar with amidochlorochloride compounds increased the pupal duration to an average 30.6and decreased in the biflorate 20.2.1days, respectively, as compared to that of control (14.4 days). These results are similar to that obtained by Abd El-Kader *et al.* (1995) who reported that larval and pupal durations of *S.littoralis* were increased due to feeding on IGRS, atabron and alsystin and their combinations. On the contrary, Ahmed (2004) mentioned that the larval period was elongated, and the pupal period shorted for the new hatched larvae of pink and spiny bollworms (Laboratory strain) treated with the higher concentrations of Spinosad when compared with untreated larvae.

2. Pupation and adult emergence:

Data represented in Tables (3 and 4) demonstrated that the treatment of the 2nd and 4th instars larvae of *S. littoralis* with the three tested compounds amidochlorochloride, lambida and biflorate compounds caused a highly significant ($p<0.01$) reduction of the pupation percentages, as compared to that of control .The 2nd larval instars treated with the lambida resulted non pupation percentage, reduction in amidochlorochloride compound the pupation ranged 15.5% for the second instar larvae while had equal higher effect 65.0 in biflorate compound, as compared to that of the check (93.0%). Also, the treatment of the 4th instar with lambida, compounds highly significant decreased in the pupation ranged 25%, while its moderate in both , amidochlorochloride, and biflorate

compounds 57.0 and 67.0%, respectively, as compared to control.

Also, the emergence of adults in the susceptible strain was highly affected by all treatments compared to that in the control. Hence, Aly *et al.* (2011) recorded that the pupation percentage and total adult emergence of 1st and 2nd instar larvae of *Sesamia cretica* (Lederer) (Lepidoptera: Noctuidae) treated with *B. thuringiensis* at the LC_{50} concentrates were was (47 and 92 %) , (94 and 100%) and (18 and 84 %), (100 and 100%) for treated and untreated, respectively.

Table (3): Effect of amidochlorochloride, lambda and biflorate on 4th instar larvae of *Spodoptera littoralis* under laboratory conditions.

Compounds	Conc-entration (%)	Mortality % after days					Developmental effect	
		2	4	6	8	10	% pupation	% moth emergency
Amidochlorochloride	0.25	15.0	33.0	50.0	68.0	70.0	20.0	10.0
	0.50	20.0	40.0	70.0	85.0	90.0	5.0	3.0
	1.00	33.0	55.0	77.0	88.0	100.0	00.0	00.0
Lambida	0.25	10.0	28.0	44.0	55.0	67.0	30.0	18.0
	0.50	18.0	38.0	66.0	81.0	86.0	10.0	7.0
	1.00	30.0	44.0	71.0	84.0	90.0	7.0	4.0
Biflorate	0.25	4.0	7.0	13.0	20.0	28.0	70.0	70.0
	0.50	8.0	12.0	20.0	25.0	29/0	71.0	7.0
	1.00	10.0	20.0	28.0	33.0	44.0	65.0	60.0
Control		0.0	0.0	1.0	3.0	0.6	93	91.4

Table (4): Effect of amidochlorochloride, lambida and biflorate on 2nd instar larvae of *Spodoptera littoralis* under laboratory conditions.

Treatment	Larval Duration (days) ± SD	Pupation%	Pupal duration (days) ± SD	% Adult emergence ±S.D
Amido chlorochloride	18.9±1.0**	15.5	30.6±2.7**	11.1± 1.6**
Lambida	17.8+3.1**	non	----------	----------
Biflorate	18±2.5**	65.0	20.2±1.4**	7.9 ±1.1*
Control	14.3 ± 1.0	93.0	14.4±0.8	9.0 ±1.4
F value	202.9	97.00	582.8	559.9
P value	0.01	0.01	0.01	0.0004
L.S.D.at.05	0.7	10.2	0.7	4.5
L.S.D.at.01	0.9	18.00	0.9	8.2

Table (5): Biological activity of Amidochlorochloride, lambida and biflorate against the 4th instar larvae of *Spodoptera littoralis.*

Treatment	Larval Duration (days) ± SD	Pupation%	Pupal duration (days) ± SD	% Adult emergence ±S.D
Amido-chlorochloride	12.8 ± 1.1**	25.0±12**	20.8 ±2.8**	15.0±1.3**
Lambida	16.5±1.3**	17±7.1**	19.0±4.5**	33.3 ± **1.1**
Biflorate	14.9±1.4. **	67±2.3**	17.5±4.1**	82 ±25**
Control	13.2 ± 1.6	93.0	14.3 ±1.1	97.0
F value	18.7	382.8	294.3	92.7
P value	0.001	0.01	0.0001	0.01
L.S.D.at.05	0.7	8.4	0.9	5
L.S.D.at.01	0.9	15.4	1.2	9.1

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