MORTALITY, MOLTING DISRUPTION AND ABNORMALITIES OF IGRS AND BIOCIEDES ON *SPODOPTERA LITTORALIS* (BOISDUVAL) (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT: To evaluate toxic and growth disruption effects of some insect growth regulators and biocides applied to control cotton leafworm, *Spodoptera littoralis* Boisd, the insecticidal activities of three IGRs, two Biocides and one Organophosphorus compound against both second and fourth instars of *S. littoralis* were assayed under laboratory conditions**.** LC_{50} values of tested toxicants with exception of Dipel 2X were applied using leaf dip technique to determine abnormalities and growth disruption of *S. littoralis*. Based on LC_{50} and LC_{90} values, the second instar larvae reflected higher level of susceptibility towards all the tested insecticides than fourth one**.** Several anomalies were recorded along the insect's life during larval, pupal and adult stages ranged from slight to severe as affected by used insecticides**.** In the light of concluded results it is obvious that insect growth regulators exhibited strong effects on *S. littoralis* larvae especially Nomolt while the biocide compound, Dipel 2x was the least effective one among all tested materials**.**

Keywords: *Spodoptera littoralis*, Insect Growth Regulators, Dipel, Malformation

INTRODUCTION

Cotton is the most important natural textile fiber in the world and considered one of the major sources of the national income in Egypt due to its excellent and incomparable technological characters, however, this plant is reliable to be attacked by several pests allover the growing stages. The genus *Spodoptera* includes many species which constitute pests of economic importance for different crops, in different countries. Undoubtedly, among more than 1300 insect species recorded on cotton, the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) is considered as the most important cotton pest that is found almost everywhere cotton is grown in Egypt (Hosny and Isshak, 1967). *S. littoralis* larvae feed mainly on leaves and stems and can seriously retard growth or reduce production of the cotton crop. During heavy infestations, however, they can also penetrate flowers and bolls, this destructive polyphagous pest causing substantial loss could result in reduction in yield up to 50% (Russel *et al.,* 1993).

For instance, insect growth regulators (IGRs) received a great attention as a hope for the future of insect control that proved to diversely affect against *S. littoralis*. IGRs cause, growth reduction, moulting inhibition, anatomical abnormalities as well as mortality, in a vast range of insect species, many of them belonging to the Order Lepidoptera. Its action depends on the insect species and on the concentration applied. Khedr *et al*., 2005

Similarly, the bio-insecticides which provide control agents equal or better than synthetic insecticides against *S. littoralis* (Salama *et al*., 1999). These classes are considered nowadays as mainly of IPM programs. Recently, Dipel 2X (*Bacillus thuringiensis*) that combines the efficacy of synthetic insecticides with reduced risk often associated with biological products, that gave a promise effect against *S. littoralis* (Raslan, 2002).

This interest in alternative pesticides resulted from the need to provide an alternative in IPM programmes to the synthetic insecticides, whose adverse effects on man, domestic animals and agroecological systems or at least to minimize its huge deleterious effects are well known (Desuky *et al*., 2012) . The growth regulatory effects of IGRs are mostly concerned with its interference in the neuroendocrine system of the insects (Wang, 2008). The main hormones involved in growth regulation in insects are ecdysone, 20-hydroxy-ecdysone (moulting hormones) and juvenile hormone (JH). They are respectively produced in the prothoracic glands and corpora allata, through stimulation of hormones secreted in the brain (Wigglesworth 1972).

This study was undertaken to assess the larvicidal and morphogenetic effects of insect growth regulators and biocide (Dipel 2x) against the lepidopterous insect *S. littoralis* (Boisd.).

Materials and methods

1. Tested compounds

1.1. Insect growth regulators

1.1.1. Nomolt® 15% Suspension Concentrate (SC).
Common name: Teflubenzuron.
Chemical name: *N*-[[(3,5-dichloro-2,4-difluorophenyl)amino]carbonyl]-2,6-difluorobenzamide**)**

Rate: 50 cm^3 / 100 L.
Action: Chitin synthesis inhibitor, affecting molting, non-systemic insect growth regulator with stomach action.
Basic product: BASF Co.

1.1.2. Mimic® 24% Emulsifiable Concentrate (EC).
Common name: Tebufenozide.
Chemical name: 3,5-dimethylbenzoic acid 1-(1,1-dimethylethyl)-2-(4-ethylbenzoyl) hydrazide.

Rate: 350 cm^3 /feddan.

Action: Ecdysone agonist which acts by binding to the receptor site of the insect molting hormone lethally accelerates molting process.
Basic product: Dow AgroSciences.

1.1.3. Runner® 24% Suspension Concentrate (SC).
Common name: Methoxyfenozide.
Chemical name: 3-methoxy-2-methylbenzoic acid 2-(3,5-dimethylbenzoyl)-2-(1,1-dimethylethyl) hydrazide.
Rate: 150 cm^3 / feddan.
Action: Second generation ecdysone agonist. Causes cessation of feeding and premature lethal molt, agonist of 20-hydroxyecdysone a key hormone in the molting process.
Basic product: Dow AgroSciences Co.

1.2. Bio-insecticides

1.2.1. Tracer®
Common name: Spinosad 24% Suspension Concentrate (SC). Tracer is comprised primarily of two macrocyclic lactones, Spinosyn A and D, secondary metabolites produced by the actinomycete, *Saccharopolyspra spinosa* under natural fermentation condition.
Rate: 50 cm^3 / feddan. (feddan = 2400m^2).
Action: Naturalyte insecticide composed of 50-95% spinosyn A and 50-5% spinosyn D, acts primarily on insect's nervous system at nicotinic acetylcholine receptor.
Basic product: Dow AgroSciences Co.

1.2.2. Dipel 2X® (6.4 % WP).
Common name: ***Bacillus thuringiensis*** subsp. *Kurstaki* 32, 000 International Units of potency per mg.
Rate: 500 gram / feddan
Action: The endotoxins functions in the alkaline guts of lepidioterous larvae, the epithelial cells of the gut are damaged, insect stop feeding because of gut paralysis and stop feeding and evantually starve to death.
Basic product: Chemical and Agricultural Products Division, Abbott Laboratories USA.

1.3. Organophosphorus insecticide

Dursban ® (48% EC).
Common name: Chlorpyrifos.
Chemical name: O, O - diethyl O- (3, 5, 6–trichloro-2-pyridinyl) phosphorothioate.
Rate: 1 liter / feddan.
Action: Organophosphorus insecticide affecting chemical transmission of nervous signal, (ChE inhibitor).
Basic product: Dow AgroSciences.

2. Rearing technique of cotton leafworm, *Spodoptera littoralis* (Boisd.)

A laboratory (susceptible) strain of ***S. littoralis*** was reared away from any insecticidal contamination at the division of Cotton Leafworm, Branch of Plant Protection Research Institute at Zagazig, Sharqia Governorate under constant conditions 27±1 °C and 70±5% R.H. to provide insects used in the present investigation according to El-Defrawi *et al*., (1964).

3. Assay of the tested insecticides against cotton leafworm, *Spodoptera littoralis* (Boisd.) under laboratory conditions

The efficiency of the different insecticides; teflubenzuron, tebufenozide, methoxyfenozide, Tracer, Dipel 2X and chlorpyrifos, were assessed against 2nd and 4th instar larvae. Serial successive concentrations of each insecticide starting with the recommended concentration were prepared using distilled water.

Disks (9 cm. diameter) of castor bean leaves were dipped in the tested concentrations for 10 seconds, left to dry and offered to larvae, which starved for 4-6 hours before treatment (Merdan, 1968). Larvae were placed into glass jars (5 pounds); each treatment was replicated 5 times (10 larvae per each). Control disks were dipped in distilled water only. The larvae were allowed to feed on treated disks for 48 hr. then on untreated ones.

Mortality percentages were recorded after 72 hr. for all insecticides except chlorpyrifos after 24 hr. Mortality was corrected according to Abbott's formula (1925). The dosages mortality regression lines were statistically analyzed by probit analysis (Finney, 1971), Toxicity Index and Relative Potency calculated according to Sun equations, (1950):

$$\text{Toxicity index} = \frac{LC_{50} \text{ or } LC_{90} \text{ of the efficient compound}}{LC_{50} \text{ or } LC_{90} \text{ of the other compound}} \times 100$$

To estimate abnormalities and deformities, each treatment and control was replicated 5 times (10 newly molted 4th instar larvae per each), larvae fed on treated castor leaves with sub lethal concentration (LC_{50}) of each treatment with the exception of Dipel 2X which used at the recommended concentration for only 48 hours and allowed to complete their life cycle to on untreated leaved to monitor resulted anomalies through larval, pupal and adult stages for all treatments

RESULTS

1.1. Susceptibility of different instar larvae of *S. littoralis* to some insecticides

The toxic effects were listed after 72 hours for all treatments with the exception of chlorpyrifos which recorded after 24 hours due to its mode of action. Data presented in Tables (1 and 2) summarized the efficacy of different tested insecticides against 2nd and 4th instar larvae except Dipel 2X which its higher concentration applied gave mortality percentages did not exceed about 30% until 5 days post treatment, The second larval instar showed higher level of susceptibility towards the tested insecticides than fourth one.

Table 1: Susceptibility of the second instar larvae of *Spodoptera littoralis* (Boisd.) to different tested insecticides.

Insecticides	LC_{50} ppm. (Lower-Upper)	LC_{90} ppm. (Lower-Upper)	Slope	Toxicity index	Relative Potency
Nomolt (Teflubenzuron)	0.204 (0.04-0.293)	2.311 (1.464-3.637)	1.216	49.02	48.5
Mimic (Tebufenozide)	9.901 (8.747-11.192)	36.447 (28.81-50.871)	0.962	1.01	1.00
Runner (Methoxyfenozide)	0.255 (0.219-0.445)	5.484 (2.107-6.225)	2.264	39.216	38.82
Tracer (Spinosad)	1.001 (0.748-1.269)	12.349 (9.378-17.532)	1.174	9.99	9.89
Dursban (Chlorpyrifos)	0.1 (0.038-0.136)	0.809 (0.611-2.594)	1.409	100	99.01

Toxicity Index and Relative Potency based on LC_{50}

Table 2: Susceptibility of the fourth instar larvae of *Spodoptera littoralis* (Boisd.) to different tested insecticides.

Insecticides	LC_{50} ppm. (Lower-Upper)	LC_{90} ppm. (Lower-Upper)	Slope	Toxicity index	Relative Potency
Nomolt (Teflubenzuron)	8.937 (3.036-11.057)	52.055 (41.445-143.901)	1.675	5.281	7.35
Mimic (Tebufenozide)	65.736 (53.074-82.747)	1000.775 (625.94-1890.80)	1.084	0.718	1.00
Runner (Methoxyfenozide)	4.27 (1.722-6.964)	153.855 (83.707-501.264)	0.823	11.054	15.39
Tracer (Spinosad)	11.16 (7.819-14.257)	158.019 (88.417-481.289)	1.113	4.229	5.89
Dursban (Chlorpyrifos)	0.472 (0.345-0.609)	6.838 (5.024-10.212)	1.104	100	139.27

Toxicity Index and Relative Potency based on LC_{50}

According to LC_{50} and LC_{90} values, chlorpyrifos was the most effective insecticide that recorded (0.1, 0.809 ppm) for 2nd instar and 4th instars respectively. Meanwhile, tebufenozide appeared to be the least effective against both tested instars that gave (9.901, 36.447 ppm) against 2nd instar and (65.736, 1000.775 ppm) against 4th one, respectively. The rest compounds gave moderate effects against both instars that manifested (0.204, 2.311 ppm) for teflubenzuron, (0.255 and 5.484 ppm) for methoxyfenozide (1.001, 12.34 ppm) for Tracer and (9.901, 36.447 ppm) for tebufenozide at LC_{50} and LC_{90}, respectively for 2nd instar larvae. As for 4th instar larvae LC_{50} and LC_{90} for methoxyfenozide, teflubenzuron and Tracer were (4.27, 153.855 ppm), (8.937, 52.055 ppm) and (11.16, 158.019 ppm), respectively, (**Tables 1 and 2**).

2.3.6. Deformations of larvae, pupae and adult malformations

Deformations of larvae, pupae and malformations of adults resulted from 4th instar larvae of *S. littoralis* fed on castor bean oil leaves treated with LC_{50} of each insecticides except Dipel 2X that treated at the recommended rate were tabulated in Tables (3-4). Deformations and malformations were recorded based on the external morphological characters and illustrated in Figures (1-4).

The larval deformations represented in Table (3) were recorded (4.00 %) for Dipel 2X, (6.00%) for Tracer, (66.00 %) for both teflubenzuron and methoxyfenozide and (52.00 %) for tebufenozide, while chlorpyrifos and control individuals did not show any larval deformations. The pupal deformations were manifested in Tables (3) and gave (2.50, 15.38, 11.11 and 10.00 %) for Dipel 2X, tebufenozide, methoxyfenozide and chlorpyrifos, respectively. Other treatments did not cause any morphogenetic effects, meanwhile, only Tracer, teflubenzuron and chlorpyrifos recorded adult malformations that reached 16.36, 20.84 and 7.31 %, respectively, Table (3).

Table 3: Deformation percentages recorded in different stages

*Insecticides	Concentration ppm	%Larval malformation	%Pupal malformation	% Adult malformation
Dipel 2X	160.00	20.00	2.50	0.00
Tracer	11.16	56.00	0.00	16.36
Teflubenzuron	8.937	66.00	0.00	20.48
Tebufenozide	65.736	52.00	15.38	0.00
Methoxyfenozide	4.27	66.00	11.11	0.00
Chlorpyrifos	0.472	60.00	10.00	7.31
Control		0.00	0.00	0.00

*All the tested insecticide concentrations at the level of LC_{50} with the exception of Dipel 2X at the recommended concentration.

Table 4: Presence of different abnormality types as affected by tested treatments.

	Types of deformities and malformations	Insecticides					
		Teflubenzuron	Methoxyfenozide	Tebufenozide	Tracer	Dipel 2X	Chlorpyrifos
Larva	Larvae could not able to discarding the old cuticle	-	-	+	-	-	-
	Precocious molt lead to production of abnormal chitin deposition	-	-	+	-	-	-
	Swollen of the posterior part of larvae abdomen	-	-	+	-	-	-
	Partially extrusion of the alimentary canal from anus	-	+	+	-	-	-
	Extrusion of molting fluid from treated larvae	+	-	-	-	-	-
	Larval-pupal intermediates	+	+	-	+	+	-
Pupa	Pupal- Larval intermediates	-	+	+	-	+	+
Adult	Adults adhering to pupal exuvium	-	-	-	+	+	-
	Adults with malformed wings ranged from slightly to severe	+	-	-	+	-	+

The different types of deformations and malformations include:

1- Larvae could not able to discarding the old cuticle, Fig. (1 a, b).
2- Precocious molt lead to production of larvae with abnormal chitin deposition, Fig. (1 c, d).

3- Swollen of the posterior part of the larvae abdomen, Fig. (1 e).

Previously mentioned types were occurred only in the case of tebufenozide.

4- Partially extrusion of the alimentary canal from anus, this type was observed with tebufenozide, Fig. (1 f, g and h) and methoxyfenozide, Fig. (1 a, b).

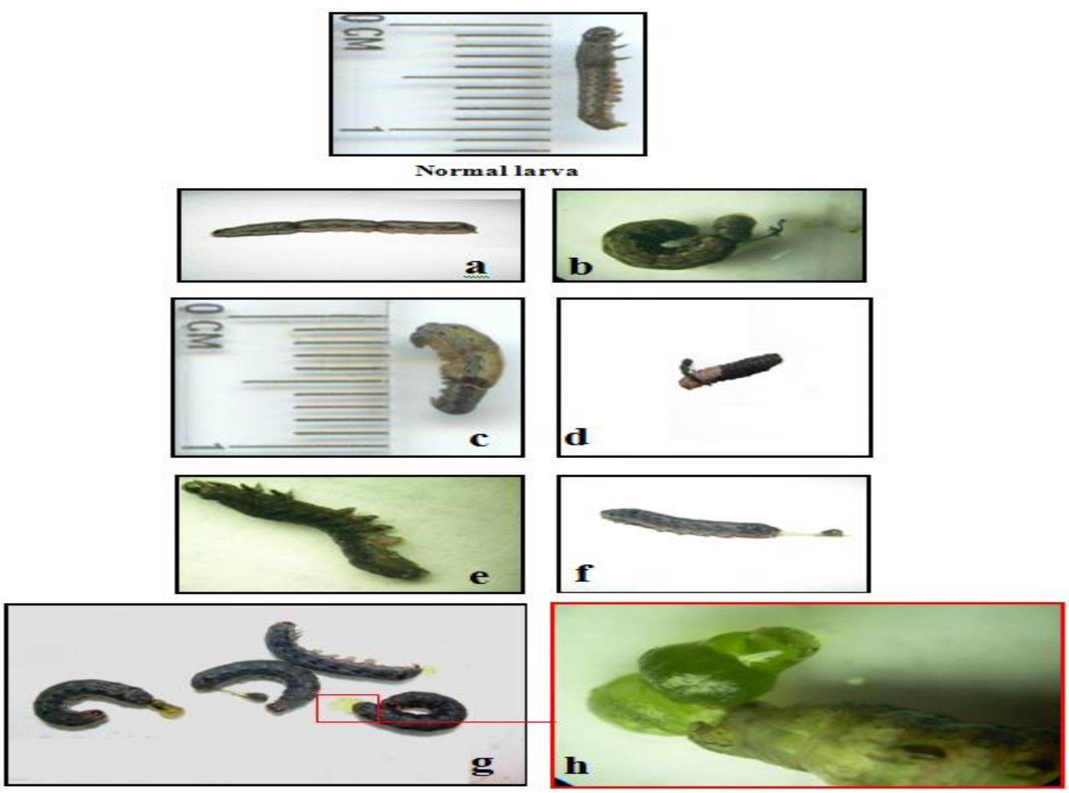
5- Extrusion of molting fluid through out different parts of larva (head thorax and abdomen) that treated with teflubenzuron, Fig. (2 c, d, e and f).

Larvae failed to pupate resulting in two types of intermediates:

6- Larval-pupal intermediates that close in shape to larva features than pupa and contains:
   - Larvae with pupal head, Fig. (3 a, b) as affected by teflubenzuron and methoxyfenozide.
   - Larvae with pupal abdomen, Fig. (3 c) after treated with Dipel 2X.
   - Larvae mouth parts attached to pupal cuticle, Fig. (3 d), this type was observed with Tracer.

On contrary, the second type of intermediates was:

7- Pupal-larval intermediates which have the external characters of pupa than larva that include:
   - Pupae with larval head that observed after treatment with tebufenozide, Fig. (3 e, f).
   - Pupae with larval head and thoracic legs that recorded by methoxyfenozide, Fig. (3 g) and Dipel 2X, chlorpyrifos, Fig. (3 h).
8- Partial emerge of adults (adults adhered to the pupal exuvium was noted when used both Trace and Dipel 2X against 4th instar larvae, Fig. (4 a, b and c).
9- Finally, adults with malformed wings ranged from slight and moderate which observed with Tracer, Fig. (4 d, e), to severe that recorded with both teflubenzuron, Fig. (4 f) and chlorpyrifos, Fig. (4g,h).



littoralis larvae with tebufenozide.

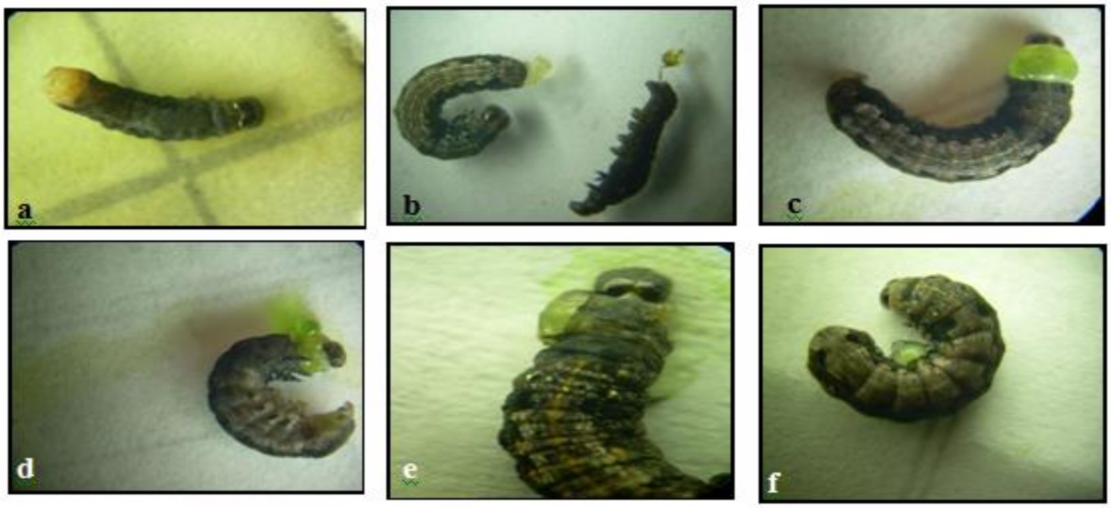


Fig. (2): Symptoms of larval mortality treated with methoxyfenozide and teflubenzuron.

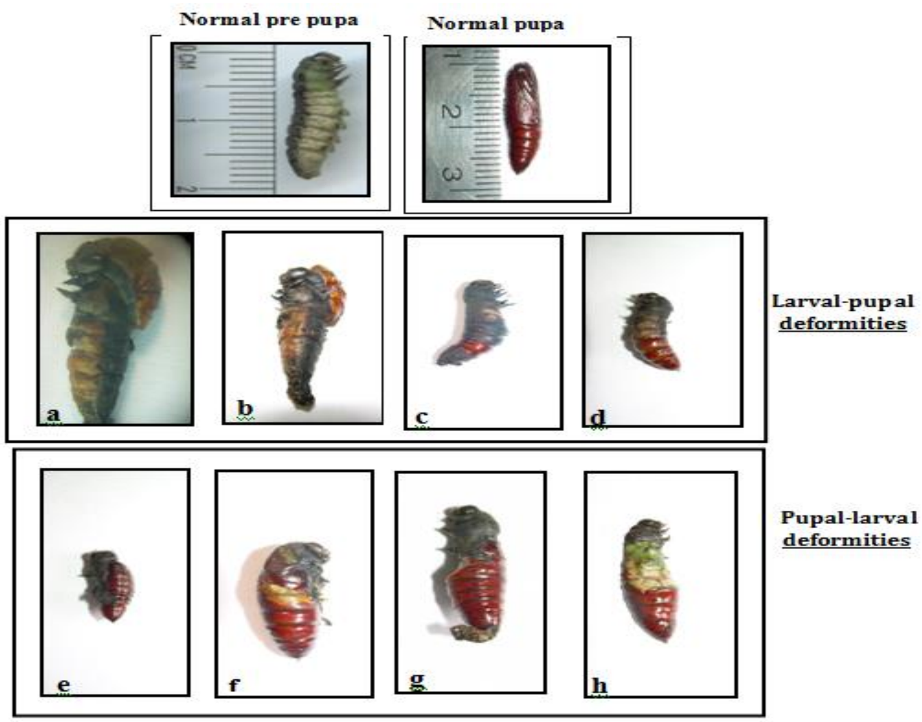


Fig. (3): Different types of intermediate individuals.

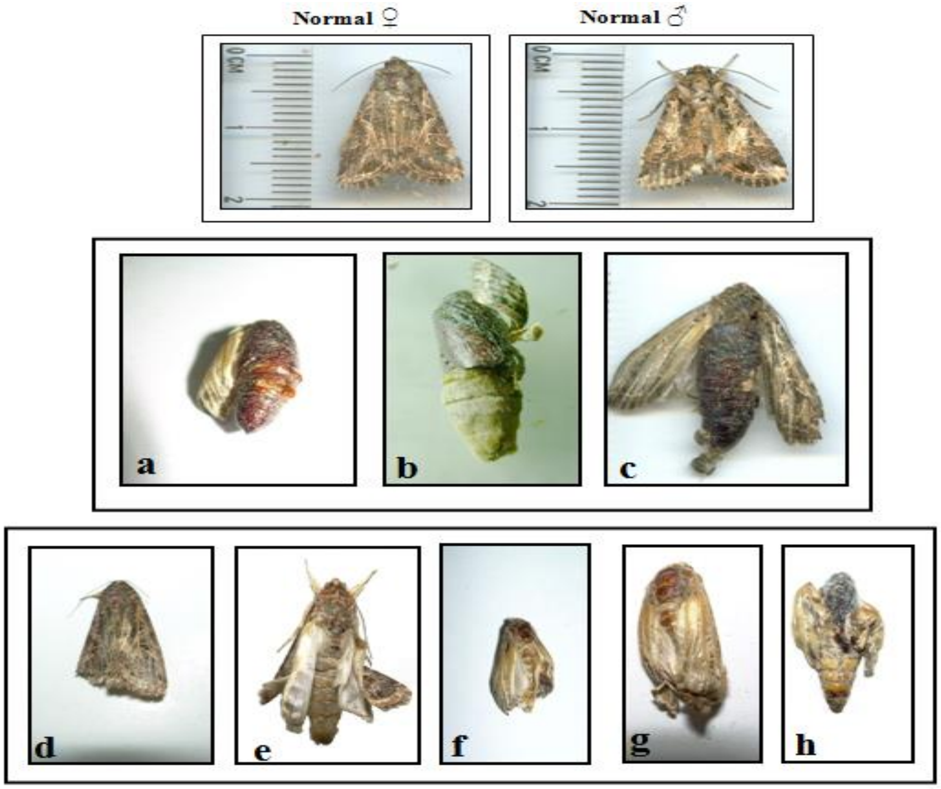


Fig. (4): Adult malformations.

DISCUSSION

According to the estimated LC_{50} values, the second instar larvae reflected higher level of susceptibility towards all the tested insecticides than fourth one. The present conclusion was in harmony with (Mead 2006) when used Consult, chlorpyrifos and Tracer against 2nd and 4th instar larvae of *S. littoralis.* Also, Abd El-Latief (2001) came to the same conclusion when tested chlorpyrifos, thiodicarb and chlorfluazuron. Romeilah and Abdel-Meguid (2000) and El-Nenaey *et al.*, (2004) found that the mortality percentages among older larvae were obviously less than among younger ones especially at the lower concentrations of five bacterial formulations. Mohamady (2000) reported that 2nd instar larvae more susceptible than 4th one to the three tested compounds, chlorfluazuron, profenofos and fenvalerate.

The higher susceptibility observed in young instars of *S. littoralis* may be contributed to the tolerance levels which were generally less than those of old ones, irrespective of IGRs tested compounds (Bayoumi *et al*., 1998). Christie and Wright (1990) suggested that the susceptibility of 5th instar larvae of *S. littoralis* more than 6th one to abamectin is due in part to greater metabolism of 5th instar than in 6th one.

On the basis of LC_{50} and LC_{90}, the present results indicated that, all the tested insecticides have larvicidal activities against both 2nd and 4th instar larvae with the exception of Dipel 2X that caused low toxic effect up to 5 days. Chlorpyrifos have the highest larvicidal and the most toxic insecticide tested against 2nd and 4th larval instars. The same result was obtained by (Ascher and Nemny, 1990 and Abd El-Latief, 2001) when tested various insecticides against eggs and larvae of *S. littoralis.* Also, El-Halim, (1993) and El-Ghar *et al.,* (1995 a) mentioned that, Dipel 2X and thuringiensin had slight or low insecticidal activities until 5 days against 2nd and 4th instar larvae of *S. littoralis.*

As for IGRs, the mortality percentages were recorded after 72 hours of treatment, because after 2 days of treatment, the IGR compounds were not effective, the mortality percentages of larvae began after 3 days of treatment (Gomaa *et al*., 1996). In addition, some IGRs have ovicidal and larvicidal activities against *S. littoralis* (El-Ghareeb, 1992 and Emam and Degheel, 1993). Pineda *et al*., (2000 and 2006) found that Spinosad and methoxyfenozide has larvicidal activities against neonates and fourth instars of *S. littoralis* under laboratory conditions.

Fourty-eight hours of feeding on castor bean oil leaves treated with the subjected insecticides; tebufenozide, teflubenzuron and methoxyfenozide resulted larvae that did not able to complete the molting process and subsequently died.

Several anomalies, possibly related to defective molting were observed as affected by tebufenozide. Larvae had problem in discarding the old cuticle or precocious molt lead to production of abnormal chitin deposition. Additionally swollen of the posterior part of larvae abdomen leading to partially extrusion of the alimentary canal from anus. The afterward symptoms were also recorded in case of methoxyfenozide. The reason of above mentioned symptoms that the origin of both fore and hind-gut is ectodermic during the embryonic development which lined with chitin layer named "intima" that consequently molt during the general molting process (Wang, 2008). Malinowski (2004) found that methoxyfenozide, teflubenzuron and diflubenzuron reacting with receptors on the brush border membrane of insect midgut, reaches the target tissue mainly by alimentary canal. The penetration of insecticides through midgut walls to haemolymph is a passive diffusion process depending on the type of compound the species of insect and its defense mechanism. Extrusion of the hindgut of lepidopteran larvae treated with methoxyfenozide and tebufenozide were observed by (Smagghe 2008).

Treatment of *S. littoralis* larvae with tebufenozide and methoxyfenozide resulted an induction of precocious and lethal molt (Carton *et al*., 2000). Furthermore, different symptoms of larvae mortality owing to feeding on leaves treated with IGR (triflumuron) were observed by (Mead, 2006), partially ecdysed larvae with big batches of new cuticle without normal coloration and larvae became inactive appeared to be wet and failed to get rid of the reminder old cuticle.

In continuity teflubenzuron, the signs of failure molting that led to mortality include the extrusion of body fluids from treated larvae during the molting process were detected. These results are in convenient with those of (Emam and Degheel 1993) who reported that ecdysis of 4th instar larvae of *S. littoralis* treated with sublethal doses of teflubenzuron to 5th instar was disrupted.

The failure in pupation process resulted intermediate individuals which can be divided into two types; larval-pupal intermediates that close to larva shape than pupa and pupal-larval intermediates which contradict with the previous mentioned type. In the same trend, (Gomaa, 2005) reported that the metamorphosis of the full-grown larvae of *S. littoralis* treated with spinosad showed failure of pupation, deformed prepupae and pupae. As well, (Moftah and El-Awami 2004) mentioned that using of chlorpyrifos and teflubenzuron on *S. littoralis* larvae produce abnormal pupae.

The latent effect of the tested insecticides hindered the development of pupae resulted from treated larvae into adults (moths) that adhering of the pupal-exuvium to the abdomen. The specific failure in adult emergence was observed by (Mohamady, 2000; Gomaa, 2005) when tested chlorfluazuron, spinosad and Dipel 2X against larvae of *S. littoralis*.

Additionally, adults with malformed wings ranged from slight to severe were also recorded. The same conclusion was obtained by (Mead, 2006) after treating 2nd and 4th instar larvae with Tracer, chlorpyrifos and triflumuron. Moreover, (Pineda *et al*., 2004 and Gomaa, 2005) found malformations in adults of *S. littoralis* as affected by spinosad.

Several anomalies and deformities reported here could be a result from the following kinds of growth disruption, which some of them involved negatively in vital activities such as feeding, walking or flying whatever make the insect easily vulnerable to several sorts of mortality agents or otherwise preventing them from causing damage to crops, therefore their injurious were stopped comparing with healthy insects.

The reason behind deformities and malformations may be a result of changes in carbohydrate hydrolyzing enzymes, chitinase, LDH and acid & alkaline phosphatases. Trehalase enzyme has the important function for liberating glucose for energy and activated during molting to generate glucose for chitin build up (Ishaaya and Ascher, 1971).also it is an important physiological process for various organisms such as insect flight and the resumption of growth in resting cells. The definition of amylase include diversified group of symptoms which may include general fatigue. So that the inhibition of carbohydrate hydrolyzing enzymes observed in the present work might affect this process. Ecdysis is initiated by apolysis, the process that separates epidermal cells from the old cuticle by molting fluid secretion and ecdysal membrane formation. Molting fluid contains protease and chitinase enzymes that digest the main constituents of the old endocuticle (Reynolds and Samuels 1996). Thus, the disruption in chitinase enzymes postulated the reason of deformations (Hegazy and Degheele 1990). In addition, elevated levels of LDH and changes in the ratio of LDH isozymes usually indicate excessive disturbance of tissues. The results obtained here consider promising for controlling *S. littoralis*, 48 hours feeding period was enough to promote morphological anomalies, moulting disruption and mortality of thus pest.

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