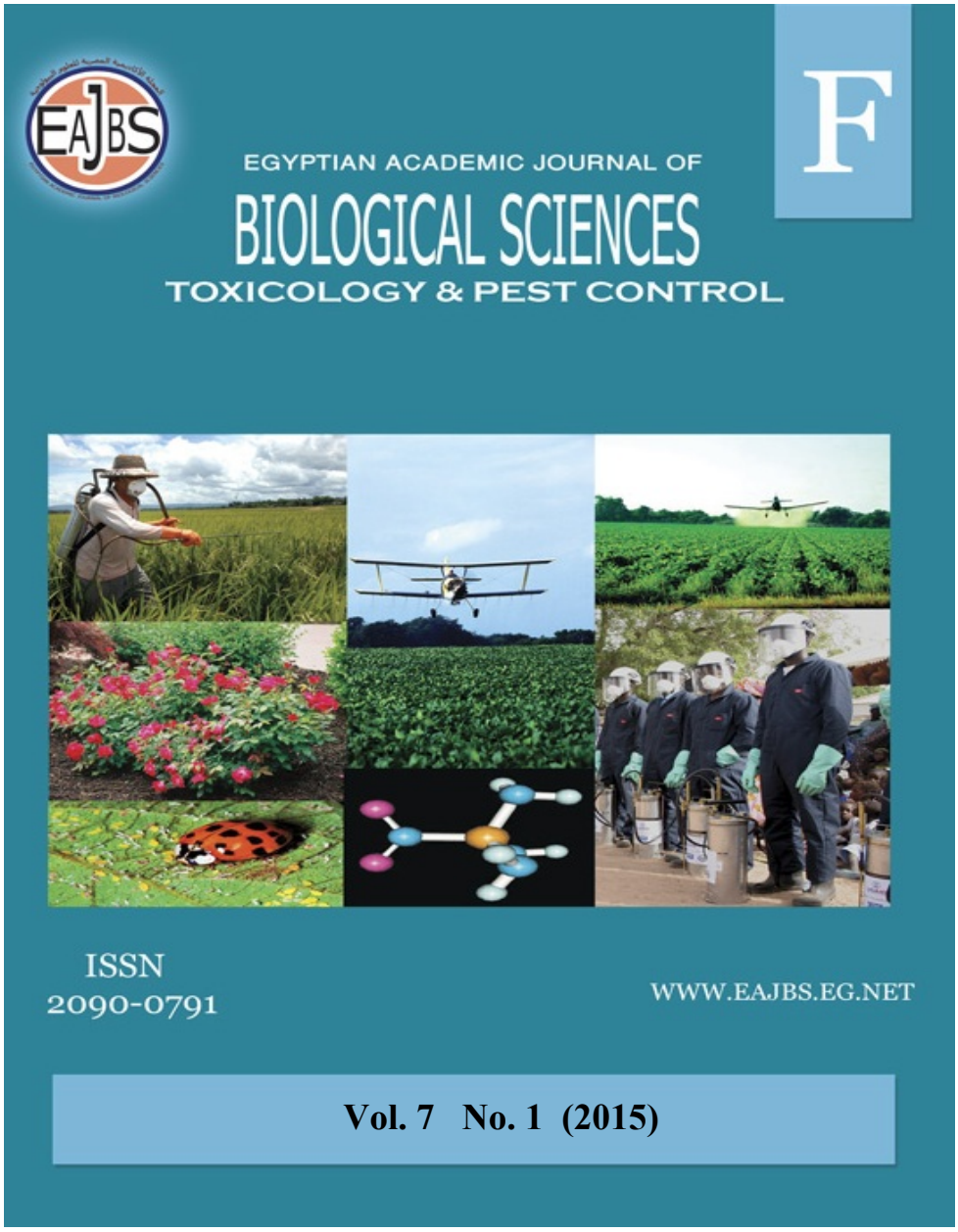

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Citation :*Egypt.Acad.J.Biolog.Sci. (F.Toxicology &Pest control) Vol.7(1)pp25-36 (2015)*

Egypt. Acad. J. Biolog. Sci., 7(1): 25-36 (2015)

Egyptian Academic Journal of Biological Sciences
F. Toxicology & Pest control
ISSN :2090 - 0791
www.eajbs.eg.net

Toxicological, biological and biochemical impacts of Indoxacarb and Methoxyfenozoid on the larvae of the Cotton leafworm, *Spodoptera littoralis* (Boisd.). (Lepidoptera: Noctuidae)

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ARTICLE INFO

Article History
Received:25/3/2015
Accepted: 18/5/2015

Key words:
Indoxacarb
Methoxyfenozoid
Spodoptera littoralis
Digestive enzymes and GOT & GPT.

ABSTRACT

The insecticidal, biological and biochemical effects of Indoxacarb and Methoxyfenozoid were evaluated on the 4th instar larvae of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). Indoxacarb exhibited high level of toxicity with Lc_{50} (0.3983ppm) followed by Methoxyfenozoid (1.7799ppm). The tested insecticides at all treatments increased larval mortality, larval duration and decreased pupation rate, pupal weight and adult emergence. Female fecundity and fertility were significantly reduced at all treatments compared to control. Furthermore, a significant reduction in the digestive enzymes (amylase, trehalase, invertase) and GOT & GPT were recorded.

INTRODUCTION

The Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisd) is one of the most notorious and destructive polyphagous insect pests in Egypt, not only to cotton, but also to other field crops and vegetables (Kandil *et al*., 2003). Over the past 25 years, the intensive use of broad-spectrum conventional insecticides against *S. littoralis* has led to the development of resistance to many registered pesticides for its control (Smagghe *et al.* 1999 and Aydin and Gurkan. 2006). In this scenario, using new types of insecticides, originated from natural agents or products that disrupt the physiological processes of the target pest, could be useful as an alternative in the integrated management approach (Dhadialla *et al*.1998, Thompson *et al.* 2000 and Smagghe *et al.* 2003). More attention should be paid to the use of novel chemical insecticides with new and different mode of action against pests and less harmful to environment (Hassan, 2009). Among these novel insecticides, Indoxacarb (Steward 15% EC), and Methoxyfenozide (Runner 24% SC).

The latest generation of insecticides includes an oxadiazine insecticide (Indoxacarb) which is active against lepidopteran pests (Wing *et al*., 1998). A previous study has shown that, *in vivo*, this compound is rapidly cleaved to its decarbomethoxyllated (the active component) appears to be a potent blocker of sodium-dependent action potentials in lepidopteran (*Manduca sexta*) larval motor nerve preparation (Wing *et al*., 1998). This mode of action differs from pyrethroids, which delay closing of sodium channels and result in the prolonged transmission of nerve signals (Soderlund and Knipple 1995).

Methoxyfenozide, an insect growth regulator (IGR), is the newest and most potent member of the moult-accelerating compounds (MACs) against Lepidoptera (Smagghe *et al.* 2003). Due to a high specificity of its action against Lepidoptera, it is considered as an environmentally friendly compound (Palli and Retnakaran 2001).

The present study was carried out to evaluate the toxicological and biological effectiveness of two novel insecticides; Indoxacarb and Methoxyfenozide on 4th larval instars of *S. littoralis* (Boisd.) under laboratory conditions and to asses biochemical impacts of Indoxacarb and Methoxyfenozide on digestive enzymes (amylase, trehalase, invertase) and Glutamate Oxaloacetate Transaminase (GOT) & Glutamate Pyruvate Transaminase (GPT) in haemolymph of the 6th larval instar of *S. littoralis* (Boisd.). These data will support insecticide use recommendations for future insecticide resistance monitoring programs.

MATERIALS AND METHODS

Test insects:-

The culture of the cotton leaf worm, *S. littoralis* (Boisd) was initiated from freshly collected eggs masses supplied from the division of cotton leaf worm of plant protection research Institute, Dokki, Egypt, formed the basis of the culture designed to provide insects used in the present work. All stages of *S. littoralis* were cultured and tested at 27±2° C and 70± 5 % R.H. Larval stages were reared on castor oil leaves which were provided daily. The formed pupae were collected and placed in clean Jars with saw dust placed at the base to provide the pupation site. Adults were provided with 10% sugar solution.

Tested insecticides.

1- Indoxacarb (Steward 15% EC, DuPont)

2- Methoxyfenozide (Runner 24% SC, Dow Agro sciences)

Bioassay of insecticide

The toxicity of the two tested insecticides was assessed on the 4th instar larvae of *S. littoralis* using leaf dipping technique. The appropriate concentration range for each compound was based on preliminary experiments. Mortality was assessed 48 hours after treatment. The larvae were scored as "affected" if noticeable paralysis was present and no movement was observed after being prodded with a brush or if they were dead. The corrected mortality of larvae was carried out using Abbott's formula (Abbott, 1925). Data then were subjected to probit analysis (Finney, 1971) for determining LC50values for each insecticide.

Biological evaluation.

Fresh castor-oil leaves were dipped for 30 seconds in each concentration of the tested insecticides (LC_{25} and LC_{50}). The treated leaves were left to dry for approximately 5 minutes at room temperature before being offered to the tested *S .littoralis* larvae for 48 hrs. The tested larvae were starved for 8 hrs. before being fed on treated leaves to ensure rapid ingestion of treated leaves. Six hrs post ecdysis, forty larvae were used, for each concentration divided into four replicates (10 larvae/ each replicate). Another group of larvae were fed on untreated leaves and kept as control. These tests were carried out to determine larval duration, percentage pupation, pupal weight, pupal duration, percentage of adult emergence, fecundity (Total number of eggs laid per female), oviposition deterrent index (O.D.I.), fertility (number of hatched eggs) and percentage of sterility.

Biochemical bioassay

Fourth instar larvae were fed on untreated or treated castor-oil leaves for 48 hours then the resulting 6th instar larvae were used for haemolymph collection. Castor-oil leaves were treated

with LC_{50} of the tested insecticides. The haemolymph was placed in 1.5ml ice-cold micro centrifuge tubes that contained few crystals of phenylthiourea (PTU) to prevent melanization. The samples were centrifuged at 2500 rpm for 5 minutes under cooling (4°C) to remove the blood cells. After centrifugation, the supernatant fluid was stored at –20°C until analysis.

Activities of the following enzymes were determined:

Activities of metabolic enzymes based on the digestion of trehalose, sucrose and starch by trehalase, invertase and amylase, respectively according to the method described by Ishaaya and Swiriski (1976). Activities of both transaminases, Aspartate transferase (AST) [also known as Glutamate Oxaloacetate Transaminase (GOT)] and Alanine transaminase (ALT) [also known as Glutamate Pyruvate Transaminase (GPT)] were determined according to the method of (Reitman and Frankel 1957).

Statistical analysis

Data from all experiments were subjected to the analysis of variance using the software computer program. The oviposition deterrent index (O.D.I) was calculated according to (Lundgren, 1975). The percentage of sterility was estimated according to the formula of (Toppozeda *et al.*,1966) Percentage of change in enzyme activity was carried out according to (Bailey, 1969)**.** Statistical significant differences between individual means were determined by one way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The results of dose-response bioassays for Indoxacarb and Methoxyfenozoid using 4th instar larvae of *S. littoralis* are summarized in (Table1). Indoxacarb exhibited significantly higher toxicity to 4th instar larvae than Methoxyfenozoid evidenced by the LC_{50} values 0.3983 and 1.7799 ppm, respectively.

Table 1: Toxicity data of Indoxacarb and Methoxyfenozide on 4th instar larvae of laboratory *S. littoralis*

Insecticides	LC_{25}ppm (95%confidence interval)	LC_{50}ppm (95%confidence interval)	LC_{90}ppm (95%confidence interval)	Slope ± SE
Indoxacarb (Steward 15%EC)	0.125 (0.115-0.135)	0.3983 (0.3370-0.4807)	2.2468 (1.5886-3.6599)	1.7058±0.1584
Methoxyfenozide (Runner 24%SC)	0.5 (0.471-0.541)	1.7799 (1.4891-2.1151)	11.2748 (8.1890-17.632)	1.5986±0.1522

Indoxacarb have been consistently reported to be highly effective against lepidopteran pests especially noctuids (Ahmad *et al*., 2003; Cook *et al*., 2004). Moadeli *et al*., (2014) reported that Indoxacarb was a remarkably potent compound for controlling the beet armyworm, *S. exigua*. Bruno *et al*., (2001) reported that Indoxacarb alters the voltage-dependent sodium channels in a manner distinct from the actions of the other compounds which effect sodium channel such as pyrethroids. It might be anticipated that a *kdr* or *super-kdr*-resistant insect would show low cross-resistance to Indoxacarb. The high effectiveness of Methoxyfenozide against Lepidopteran pest has been widely recognized (Moulton *et al*. 2002, Smagghe *et al*. 2003, and Pineda *et al*. 2004).

Biological impacts of Indoxacarb and Methoxyfenozide on 4th instar larvae of *S. littoralis*

In the present study, treatment of the 4th instar larvae of *S. littoralis* with subleathal doses of Indoxacarb and Methoxyfenozoid not only exerted their action as larval death but they also caused an extended effect which was

observed as pupal death, failure of emergence and even some adult death or malformations in wings and appendages.

Data in Table 2 show that the treatment of the 4th instar larvae of the *S. littoralis* with both tested insecticides resulted in a significant prolongation in the larval duration. This prolongation was more obvious with Indoxacarb treatments than Methoxyfenozoid and at LC_{50} values than with LC_{25} values.

Table 2: Effect of Indoxacarb & Methoxyfenozoid on biology of 4thinstar larvae of *S. littoralis*

Insecticides	Dose	Larval duration (days) (Mean ±SE)	Pupation %	Pupal weight (mg) (Mean ±SE)	Pupal duration (days) (Mean ±SE)
Indoxacarb	Lc_{25}	12.25±0.48	77.5±2.50	0.30±0.01	10±0.41
	Lc_{50}	14.50±0.96	45.0±2.89	0.29±0.00	9±0.00
Methoxyfenozoid	LC_{25}	10.50±0.65	77.50±2.50	0.3125±0.01	11.00±0.91
	LC_{50}	11.75±0.25	55.00±2.89	0.2950±0.00	12.5±0.87
Control	0.0	10.00±0.41	100±0.00	0.36±0.02	10.5±0.29

Indoxacarb showed feeding inhibition at all concentrations as it act as antifeedant the same was also as reported by (Hassan, 2009). A symptom of toxicity as a result of treatment with this chemical was evident with tremors of the larval thoracic legs and mouth parts. Onset of tremors has resulted in insects were unable to feed (Wing *et al*., 2000; Bostanian *et al*., 2004). Therefore, feeding impairment of treated larvae has lead to prolongation of the larval instars and subsequently reduction in the percentage of pupation and adult emergence. Similar observation was also reported by (Vishal *et al*., 2005 and Singh and Sohi, 2008) on *S. littoralis* by Indoxacarb. Prolongation of larval stage after treatment of *S. littoralis* larvae with sublethal concentrations of Methoxyfenozoid have also reported by (Knight, 2000 and Pineda *et al.,* 2004, 2007). Similar observation in *Agrotis ypsilon* has also been reported by (Fahmy, 2014).

The percentage of pupation resulted from treatment of 4th instar larvae with LC_{50} of both tested insecticides was reduced compared to the control insects (Table 2). This reduction has reached about 55% and 45% with Indoxacarb and Methoxyfenozoid, respectively as compared to control insects.

Moreover, Table (2) shows that both tested insecticides caused a reduction of the mean pupal weight of pupae resulted from treated larvae. Again this reduction was more obvious with LC_{50} than LC_{25} treatments. This reduction has reached 20% less than control insects with both compounds. This reduction in weight was most probably due to the loss of appetite of the larvae 48-hours post treatment with insecticides and due to decrease of food intake by the larvae. These results were promising since adult population suppression of the next generation would be achieved. These adults will not be able to tolerate any adverse environmental conditions due to the reduction in their weight and inhibition of protein, lipid and carbohydrate (Hassan *et al*, 2014). Wanner *et al*., (2000) and Al Shannef *et al.,* (2006) also reported that mature larvae either died as prepupae or as an intermediate between larvae and pupae. The reduced number of larvae entering pupation or moth emergence could be a result of accumulation of toxic material in the insect's body.

Results shown in Table 2 indicate that Indoxacarb has decreased the mean pupal duration of the *S. littoralis* pretreated as 4th instar larvae. This reduction has reached 15% as compared to the control. On the other hand,

Methoxyfenozoid has increased the mean pupal duration of the *S. littoralis* pretreated as 4th instar larvae. This prolongation has reached about 25% as compared to control.

These results were in accordance with the finding of (Fahmy, 2014). This can be probably be attributed to the hormone mimic action of Methoxyfenozoid (Dhadialla *et al*., 1998).

The percentage of adult emergence resulted from pretreated 4th instar larvae has been highly reduced. Emerged adults derived from treated larvae with Indoxacarb and Methoxyfenozoid LC_{50}s were 62.5% and 55% less than control insects, respectively (Table3). This reduction was more obvious with LC_{50} than LC_{25} treatments.

These results were in accordance with the findings of (Enriquez *et al*., 2010) on *S. exigua*. The decrease in adult emergence could be due to the effect of the tested insecticides which block the maturation of imaginal discs which are primordial for many adult integument structures in endopetrygote insect (Charles *et al.,* 2000**)**. The same results were also in accordance with the findings of Reda *et.al*. (2010) who reported that flufenoxuron increased the larval and pupal duration and decreased the pupation, adult emergence and fertility of the eggs produced by adult progeny of *S. littoralis*.

A significant reduction in the (fecundity) number of eggs laid per female derived from treated 4th instar larvae has resulted (Table 3). This reduction reached about 65% and 50% with Indoxacarb and Methoxyfenozoid Lc_{50}s, respectively as compared to control insects. The reduction might either be due to the direct interference with the hormonal system or loss of appetite due to insecticidal poisoning, and which in turn affected the hormones and thus directly affected the fecundity of the insect.

Table 3: Effect of Indoxacarb & Methoxyfenozoid on emergency, fecundity, fertility and sterility of adult derived from treated 4th instar larvae of *S. littoralis*

Insecticides	Dose	Adult emergence %	Total Inhibition of Adult Emergence %	Number of eggs/ Female Mean ±SE	Egg hatching % (Mean± SE)	O.D.I. %	Sterility %
Indoxacarb	Lc_{25}	67.5	32.5	372.75±1.65	22.47±1.66	41.52	90.50
	Lc_{50}	37.5	62.5	320.75±10.36	5.32±1.04	47.53	98.06
Methoxyfenozoid	Lc_{25}	72.5	27.5	499.50±3.01	63.37±0.65	28.72	64.11
	Lc_{50}	45	55	433.67±2.52	46.24±1.55	35.06	77.26
Control	0.0	100.0	0.0	902.00±1.58	97.79±0.26	0.0	0.0

Table 3 shows that hatchability of eggs laid by females derived from treated larvae was significantly reduced. Hatchability% ranged from 5.32 - 46.24% with Indoxacarb and Methoxyfenozoid LC_{50}s compared to 97.79% for control tests.

The oviposition deterrent index (O.D.I) and sterility% were more obvious with Indoxacarb treatments than Methoxyfenozoid and at LC_{50} values than with LC_{25} values.

Reduction in fecundity noticed in this study was also reported by several investigators (Pineda *et al*., 2007; Rastegari and Subrahmanyam, 2008; Pineda *et al.,* 2009; Enriquez *et al*., 2010; Shahout *et al*., 2011; and Zarate *et al*., 2011) on *Spodoptera* species. Acheuk *et al*., 2012 suggested moreover, that chitin deficiency weakened the exoskeleton and muscle attachment in the embryo, rendered it incapable of withstanding the strong pressure required for successful hatching, thus reduced hatching of eggs laid by treated females.

Activity determination of various enzymes in haemolymph of treated

and untreated 6th instar larvae of S. *littoralis*.

The utilization of main nutrients depended on the digestive enzymes; amylase, trehalase, invertase (carbohydrate enzyme). Generally, insect digestive enzymes could be used as a parameter for determining feeding and growth deterrent activity of certain compounds (Ishaaya *et al*., 1971). Three digestive enzymes; were determined in haemolymph of treated 6th instar larvae of *S. littoralis* resulted from treated 4th instar larvae with LC_{50} of the tested insecticides; Indoxacarb and Methoxyfenozide.

Data in (Fig. 1) showed that treatment with both Indoxacarb and Methoxyfenozide caused considerable reduction compared to the control in amylase; invertase and trehalase activity. The disturbance in carbohydrate was expressed by impairments in the activity of carbohydrate enzymes in treated larvae. Similar observation in *S. littoralis* has also been reported by several investigators (Radwan *et al*. 1984; Eid, 2002; El-Barky *et al*., 2008; Dahi *et al*., 2009 and Rashwan, 2013).

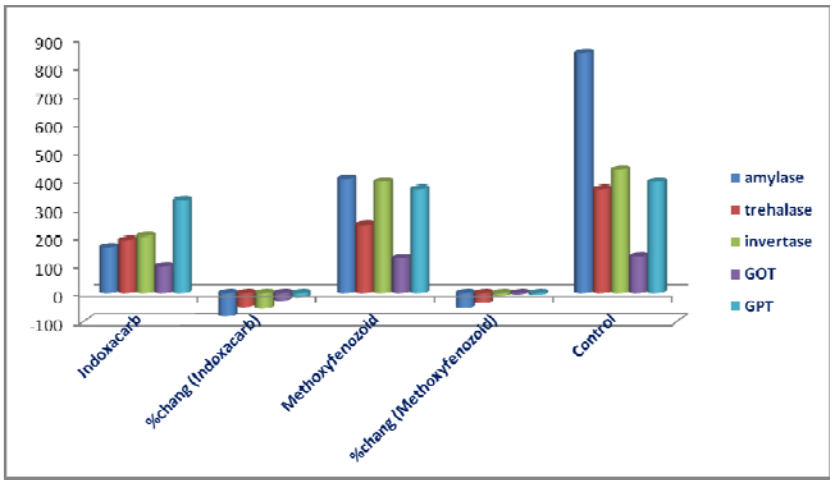

Fig. 1: Activity of digestive enzymes and transaminases (GOT & GPT) in haemolymph of treated and untreated 6th instar larvae of S. littoralis.

In insects, as in other animals, the transaminases (GOT and GPT) are key enzymes in the formation of non-essential amino acids, in metabolism of most nitrogenous products and in glucogenesis (Tanani *et al*., 2009).

It was cleared from results in (Fig. 1) that treatment of *S. littoralis* larvae with Indoxacarb and Methoxyfenozide caused significant decrease in the transaminases activity than that in untreated larvae. Increased transaminases levels had been correlated with protein anabolism in some instances and with protein catabolism in some others (Chen, 1966). These changes were in agreement with that recorded by **(**Abd El-Mageed and Elgohary, 2006 and Hanan, 2014). Abdel Hafez *et al*., (1993**)** reported that treatment of *S. littoralis* larvae with Cyanophos, Methomyl and 2 IGR_s and their mixtures caused variable reduction in GOT and an increase in GPT activity compared with control. Also, GOT in field and laboratory strains of *S. littoralis* treated with six IGRs were inhibited by (-3.69 and -28.14%), respectively, GPT as well was inhibited in both strains and the inhibition ranged between (-13.85 and -42.17%) (Anwar and Abdel-Mageed, 2005). The reduction in GOT and GPT activities due to larval treatment with Indoxacarb and Methoxyfenozoid could be attributed to the hormonal control of

protein synthesis and transaminases (Etebari *et al.,* 2005).

Malformations of 4th instar larvae of *S. littoralis* due to treatment with Methoxyfenozoid.

Treatment of 4th instar larvae of *S. littoralis* with different concentrations of Methoxyfenozide resulted in different malformations included the formation of larval-pupal intermediates (Fig.3-A), deformed pupae with larval mouth (Fig.3-B), deformed adult moths with short and curled wing (Fig.3-C) as compared to the control (Fig. 2 A, B, and C). Malformation following IGR exposure has been reported by Nomura and Miyata, (2000) who recorded that female adults of S. *litura* treated with Pyriproxyfen showed wing abnormalities. The disturbance of trehalase activity might hamper the supply of glucose needed for chitin build up (Candy and Kilby, 1962). Also Dhadialla *et al*. (1998) reported that Methoxyfenozoid mimics the natural insect molting hormone by binding competitively to ecdysteroid receptors in insect cells, thus inducing a premature larval molt.

Finally, it could be concluded that Indoxacarb and Methoxyfenozoid represent an important choice to be used in integrated pest management where *S. littoralis* is a major pest to minimize adverse effects of the traditional chemical insecticides on the environment as well as beneficial insects. In addition, we demonstrated that Indoxacarb and Methoxyfenozide affected the reproduction of this pest. These effects are important from a practical point of view, because offspring can then be reduced and as a consequence, the insect population density can be maintained below the economic threshold.

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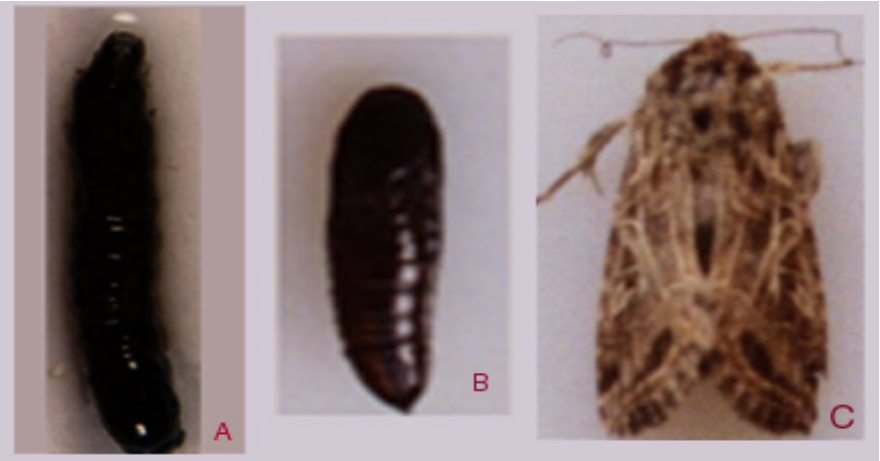
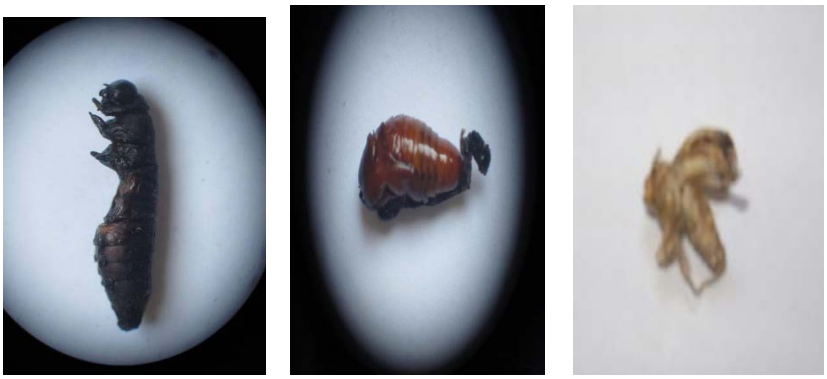


Fig. 2: Normal morphology of *S. littoralis* (larva, pupa and adult).
A- Normal 6th instar larva.
B- Normal pupa.
C- Normal adult.



(A) (B) (C)

Fig. 3: Morphological abnormalities produced by Methoxyfenozoid:
A- larval-pupal intermediates.
B- deformed pupae with larval mouth.
C- deformed adult moths with short and curled wing.

ARABIC SUMMERY

التأثيرات السمية والبيولوجية والكيموحياتية للاندوكساكارب والميثوكسيفينوزويد على دودة ورق القطن سبودوبتيرا ليتوراليز (حرشفية الأجنحة – نوكتويدي)

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تم تقييم المبيدات والأثار البيولوجية والبيوكيميائية للاندوكساكارب والميثوكسيفينوزويد علي العمر اليرقي لدودة ورق القطن سبودوبتيرا ليتوراليز (حرشفية الأجنحة – نوكتويدي). أظهر الاندوكساكارب مستوى عالي من السمية اعتمادا على قيم التركيز نصف المميت Lc_{50} (٠.٣٩٨٣) يليه الميثوكسيفينوزويد (١.٧٧٩٩) . في كل معاملات المبيدين المختبرين ازداد معدل وفاة اليرقات ، عمر اليرقات ، انخفاض نسبة التعذر ، وزن العذارى وخروج الحشرة الكاملة. أنخفضت ايضا خصوبة الاناث ونسبة فقس البيض انخفاضا ملحوظا في كل المعاملات مقارنة بالحشرات غير المعاملة . وعلاوة على ذلك سجلت مستويات مختلفة من تغيرات ملحوظة في الإنزيمات الهاضمة الأميليز ، التريهاليز ، الإنفرتيز و (GOT and GPT)