Insecticidal effects of azadirachtin as a new approach for integrated management of *Spodoptera littoralis.*

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ABSTRACT: The efficiency and toxicity of azadirachtin was studied against second and fourth larval instars of the cotton leafworm, *Spodoptera littoralis*, that have been collected from heavily sprayed cotton fields with conventional insecticides at Abou El-Matamir location, (EL-Boheira, Governorate), Egypt. The results exhibited that the second instar larvae was more susceptible than the fourth instar larvae. Increasing the concentration caused an increase in mortalities among larvae of both instars. The LC_{50} values of azadirachtin decreased by increasing the post treatment period. The LC_{50} values after 2 days were >20.0 and >20.0 ppm for 2nd and 4th instar larvae, respectively, while LC_{50} values were 17.2 and >20.0 ppm, after 4 days respectively. The LC_{50} values reached 4.3 and 8.6 ppm, after 9 days respectively, while they were 1.1 and 3.3 ppm, respectively after 12 days. The latent biological effect of azadirachtin caused different effects including stop of feeding, inhibition of pupation, formation of intermediates and malformed pupae and adults, but the ratio of malformed pupae and intermediate were higher in case of the fourth instar larvae. The symptoms of toxicity included stop of feeding, delay or prevention of pupation, blackening the body, failure of molting to the next larval instar, formation of larval-prepupal intermediates and malformed pupae, where the intensity of symptoms was proportional to concentrations. Effects normally indicated that, azadirachtin is a successful insecticide that may be utilized in incorporated pest management applications of the cotton leafworm to save you or put off the advent of resistance to traditional pesticides

Keywords: azadirachtin, physiological aspests, biological aspects, *Spodoptera littoralis*

1.INTRODUCTION

The rich source of novel natural substances, which can be used to develop environmental safe methods for insect control, are higher plants. Plants that contain different biochemical components of which many have some potential effects and can be used for controlling pests and diseases. Using plant-derived compounds to control pests has gained increasing importance over conventional insecticides because of their excellent biodegradation in addition to their safety to humans, hot blooded animals and natural enemies of insects, in addition to their unstable nature pests **(Warthem *et al*., 1978; Warthem, 1979).** In recent years there were reports about many plants with active properties that can be used as pesticides against several insect pests **(Carlini and Grossi, 2002; Kundu *et al*., 2007).**

One of the most harmful insect pests is cotton leafworm, *Spodoptera littoralis,* which attacks a wide range of economic plants. The problems of this insect is not only due to its direct damage to the infested plants, causing greet losses in their yield but also to quality and the losses extended to oil content in the seeds. Chemical control is still considered one of the most important methods for controlling the cotton leafworm, but many problems arose from using conventional insecticides **(Metcalf 1980).** Therefore, many efforts were directed to use natural plant products as insect toxicants, antifeedants, and oviposition deterrents. Therefore, biopesticides are considered one of the promising tactics in insect-pest management **(Freeman and Andow, 1983; Klocke, 1987; Srivastava *et al*., 1990; Hough-Goldstein and Hahn, 1992).**

Thus, the aim of the present study was to evaluate the efficacy of natural plant products such as azadirachtin against different larval instars of the cotton leafworm, *S. littoralis*.

2.MATERIALS AND METHODS

2.1. Test insects:

Field strain of the cotton leafworm, *Spodoptera littoralis* egg masses were gathered from cotton fields at Abou El-Matamir (EL-Boheira, Governorate), Egypt. Experiments were carried out using both second and fourth larval instars, which were chosen for bioassays.

2.2. Test compound:

Azadirachtin, Neemix 4.5, a 4.5 % azadirachtin EC formulation, was purchased from Thermotrilogy, USA.

2.3. Bioassay tests:

2.3.1. Toxicity of the azadirachtin against *S. littoralis* larvae:

The testes were carried out using a-day-old second and fourth instar larvae, for the bioassay. Various concentrations of azadirachtin 2.5; 5; 10; 15, and 20 ppm were prepared by diluting the commercial formulation with water. Castor bean leaf discs, which are 7cm in diameter, were dipped in different prepared concentrations of azadirachtin for about ten seconds and left to dry, before they were offered to the larvae to feed on for two days in Petri dishs containing ten (2nd or 4th instars) larvae of *S. littoralis* and then untreated fresh discs were offered to feed on. Similar numbers of larvae were fed on castor bean leaves discs dipped in water and served as control. Five replicates for each concentration were used. Mortality percentages were recorded after 2, 4, 9, and 12 days. According to **Finney (1971),** probit analysis of results was carried out after correcting the mortality in according to **Abbott's formula (1925)** due to control mortality. The pupation percentage was tallied. Alive larvae were maintained in Petri dishes underneath laboratory conditions of 27 ± 2°C and 65 ± 5 %

RH and supplied with untreated castor leaves. Mortality counts was recorded daily till pupation. The emergent pupa was kept in Petri dishes till adult emergence. Percentage of pupation and grownup emergence was calculated.

3.RESULTS AND DISCUSSION

3.1.Toxicity of azadirachtin against *S. littoralis* larvae:

Data in Table 1 showed the mortality percentages in addition to LC_{50} values of the second and fourth larval instars of *S. littoralis* at sequence periods after application with azadirachtin. The results exhibited that the second instar larvae were more susceptible than the fourth instar at all tested concentrations. The mortality death rate of larvae increased as the concentration of azadirachtin increased. The LC_{50} values were >20.0 and >20.0 ppm after 2 days for 2^{nd} and 4^{th} instar larvae respectively, while LC_{50} values were 17.2 and >20.0 ppm, after 4 days respectively. LC_{50} values were 4.3 and 8.6 ppm, after 9 days respectively, LC_{50} while these values were 1.1 and 3.3 ppm, after 12 days respectively. The larvae fed on azadirachtin-treated leaves stopped feeding after two-four days. Their colour changed gradually into black, prolongation of the larval instars was noticed, failure of the molting process and formation of morphological anomalies increased with the increase of the concentrations, but control larvae developed normally to the pupal stage after 9-11 days, while treated larvae remained inactive for several days (sometimes to 14 days). The present results are confirmed by saveral of investigators **(Blaske and Saxenal, 1990; Ascher, 1993; Benner, 1993; Shapiro *et al*., 1994; Eeswara *et al*., 1998; Mordue and Nisbet, 2000; Martinez and Van-Emden, 2001; Babu and Nair, 2004; Babu *et al*., 2006; Jabilou *et al*., 2006).**

larvae the formation percents were 43.61; 31.73; 17.56, and 6.85 % at the same concentrations, respectively. The results were in comparison with control. The ratio of malformed pupae and intermediates were higher in case of the fourth instar larvae. The adults appeared with malformed wing or wingless, or with only one pair of the wings.

It could be that azadirachtin has an antimoulting action where the exuvia were attached to the new cuticle. The last larval instar as well as the pupal stage failed to moult and thus larval-pupal and pupal-adult intermediates; such individuals soon die during few hours. Similar obsservations were found by **Harwood *et al*. (1990).** The effect of azadirachtin on the larval instars and on the developmental stages of *S. littoralis* may be due to the inactivation of juvenile hormone (JH) by altering the microsomal cytochrome P -450 oxidase system and or by the mimic of JH action that induces malformations **(Beckage *et al*., 1988).** Therefore, utilization of such material as alternative insecticides for insect control programmes should be considered. These finding have been confirmed by the results of many authors such as **Allan *et al*. (1994), Rice and Coats (1994), DiCosmo and Misawa (1995), Jilani and Hertel (2001), Kraus (2002), Sarlini and Grossidi (2002), Onyilagha *et al*., (2004); Sujanya *et al*. (2008).**

In conclusion, the present investigation proved that azadirachtin is a successful botanical insecticide, that could be used in the integrated management programs to control *S. littoralis* to prevent or delay the appearance of resistance to conventional insecticides, however, it is better to use azadirachtin in sequences with other insecticides because resistance to azadirachtin is not possible after several applications of the compound because it has multi effects on insects, and there is more than one target for the compound in insects to be affected.

Table (1), Mortality percentage of azadirachtin on *S. littoralis* larvae

Conc. (ppm)	Mortality percentage post treatment (days)							
	2		4		9		12	
	2^{nd}	4^{th}	2^{nd}	4^{th}	2^{nd}	4^{th}	2^{nd}	4^{th}
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2.5	20.04	21.23	25.41	22.22	40.96	28.77	61.85	45.66
5	25.13	27.04	30.22	27.31	53.77	39.56	70.78	57.97
10	38.11	33.33	40.20	35.44	67.75	51.85	91.66	70.68
15	44.43	39.02	49.43	40.10	72.69	60.99	100.0	95.11
20	48.21	43.12	52.44	46.31	100.0	94.67	100.0	100.0
LC_{50}	>20.0	>20.0	17.2	>20.0	4.3	8.6	1.1	3.3

3.2.Latent biological effect:

Results in Table 2 showed the toxic effect of azadirachtin on the developmental stages of *S. littoralis* at tested concentrations of azadirachtin, which showed that the percentages of adult emergence were less than that in the pupal stage. The adult emergence percentages were 11.23 and 20.41 % by 2.5 and 5.0 ppm of azadirachtin against the second instar larvae, wherease, they were 17.56 and 32.78 % on the fourth instar larvae. On the other hand, malformed pupae and adults with 31.02; 17.14, and 4.32 % caused by 2.5; 5.0, and 10 ppm of azadirachtin on the second instar larvae, respectively, wherease, in case of the fourth instar

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Table (2), Latent effects of azadirachtin on *S. littoralis* larvae

Conc.(ppm)	% pupation		% emerged adult	
	2nd	4th	2nd	4th
Control	100	100	100	100
2.5	31.02	43.61	11.23	17.56
5	17.14	31.73	20.41	32.78
10	4.32	17.56	0.0	0.0
15	0.0	6.85	0.0	0.0
20	0.0	0.0	0.0	0.0

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التأثيرات الأبادية لمادة الأزديراختين كأتجاه حديث للمكافحة المتكاملة لدودة ورق القطن.

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تم دراسة تأثير النشاط الأبادى وسمية مادة الأزديراختين على العمر اليرقى الثانى والرابع للطور لحشرة دودة ورق القطن تركزت الدراسة على العشائر المنتشرة فى المناطق التى ترش بمعدل كثيف من المبيدات التقليدية (أبو المطامير محافظة البحيرة - مصر). أوضحت النتائج أن الأزديراختين أظهر سمية عالية تجاه العمر اليرقى الثانى والرابع لدودة ورق القطن وكانت يرقات العمر الثانى أكثر حساسية من يرقات العمر الرابع، حيث كانت قيم التركيزات اللازمة لموت 50 % من اليرقات أكبر من 20.0 و 20.0 جزء فى المليون بعد يومين من المعاملة وذلك بالنسبة ليرقات العمر الثانى والرابع على التوالى بينما بعد 4 أيام من المعاملة كانت هذه التركيزات 217. و أكبر من 20.0 جزء فى المليون على التوالى وكانت 3.4 و 6.8 جزء فى المليون بعد 9 أيام من المعاملة على التوالى بينما كانت 1.1 و 3.3 جزء فى المليون بعد 12 يوم من المعاملة على التوالى. ومن النتائج أيضاً يتضح أن الأزديراختين له تأثير على تحول الطور اليرقى لطور ماقبل العذراء حيث سببت بعض التركيزات تشوهات فى طور ماقبل العذراء وكذلك تشوهات فى طور العذراء وكذلك للطور الحشرى الكامل الناتج عن المعاملة بالأزديراختين. وقد أدت المعاملة بالأزديراختين إلى عدة تاثيرات تضمنت التوقف عن التغذية وتثبيط تطور اليرقات إلى العذارى كما أدت إلى تكون أفراد غير مكتملة النمو من العذارى وكذا من الحشرات الكاملة. والنتائج بصفة عامة توضح أن الأزديراختين يمكن أن يستخدم كمبيد حشرى ضمن برنامج المكافحة المتكاملة لدودة ورق القطن وبذلك نتفادى زيادة مقاومة هذه الآفة بفعل المبيدات التقليدية.