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Insecticidal effects of azadirachtin on the cotton leafworm, *Spodoptera littoralis* (Boisd)

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ABSTRACT

The effects of Neemix 4.5 % (4.5 % azadirachtin) on different larval instars of the cotton leafworm, *Spodoptera littoralis,* were studied by feeding the larvae on castor bean leaves treated with different concentrations of azadirachtin. The second instar larvae were the most sensitive amongst the three larval instars exposed to various treatments. The concentrations that caused 50 % reduction in pupation of the second, third and fourth larval instars were 3.67, 10.75 and 10.92 ppm, respectively. Azadirachtin caused different effects including stop of feeding, inhibition of pupation, formation of intermediates and malformed pupae and adults. The compound showed contact toxicity when it was applied topically to the larvae and contact toxicity was much less than feeding toxicity. Topical application treatment caused same effects of feeding toxicity. The treatments caused reduction of egg mass production in a concentration-dependent manner. However, azadirachtin did not prevent *S. littoralis* females from oviposition on treated plant leaves. The results generally indicate that azadirachtin is a successful insecticide, which may be used in integrated pest management programs to prevent or delay appearance of resistance to conventional pesticides.

INTRODUCTION

The intensive use of conventional pesticides cause many environmental problems. They accumulate in food chains and pollute soil, water and air. They also lead to the destruction of natural enemies and to the appearance of the problem of pest resistance to pesticides. The cotton leafworm, *Spodoptera littoralis* is a very destructive pest, it attacks a wide variety of field crops and causing great economic losses. This pest rapidly acquires resistance to all classes of applied insecticides. *S. littoralis* has developed resistance to organophosphorus insecticides (Ishaaya and Klein, 1990), to pyrethroids (Issa *et al.*, 1984 and Ishaaya and Klein, 1990), to urea derivatives (Keddis *et al.*, 1988), to carbamates (El-Sayed and Abdallah, 1988), and even to the biological insecticide, *Bacillus thuringiensis* (Chaufaux *et al.*, 1997 and Salama *et al.*, 1989). El-Sebae (1977) indicated that *S. littoralis* has possessed resistance nearly to all registered insecticides in Egypt.

The promising botanical compound, azadirachtin, extracted from the Indian neem tree, *Azadirachta indica*, proved to be effective against a large number of pests from different orders (Immaraju,1998). Extracts of seeds of *A. indica* and commercial formulations containing different concentrations of azadirachtin, have been tested against *S. littoralis* (Hashem *et al.*, 1998; Martinez *et al.*, 2001; Meisner and jemny, 1992; El-Sayed, 1985). The present investigation presents some promising results of the effects of azadirachtin on different larval instars of *S. littoralis*.

MATERIALS AND METHODS

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

***Spodoptera littoralis*:** A susceptible strain of *S. littoralis* reared in the insectary of Plant Protection Department, on castor bean leaves, in 1-liter glass jars covered with muslin, was used in this study. In case of using replicates with relatively high number of larvae (50 larvae/ replicate), 3-liter glass jars were utilized.

The leaf –dipping method: Different concentrations of azadirachtin were prepared by diluting the commercial formulation, Neemix 4.5, with water. Fresh leaves of castor bean were immersed in different concentrations of azadirachtin for about ten seconds. The leaves were air- dried, by hanging in air, before offered to the larvae to feed on for two days and then replaced with fresh, untreated leaves. Control leaves were immersed in water. Twenty, thirty and fifty larvae were used for every replicate in trials of the second, third, and fourth instar larvae, respectively. Three replicates were used for each concentration. Probit analysis of results was carried out according to Finney (1971) after correction for mortality in controls according to Abbott's formula (1925).

Topical application trials: Different concentrations of azadirachtin were prepared by diluting Neemix 4.5 with acetone; one microliter of each concentration was applied on the dorsum of fourth instar larvae, weighing approximately 44 mg pear larva. The doses ranged from 0.25 to 4 micrograms/larva. Controls were treated with one microliter of acetone, fifty larvae were used in every replicate, and three replicates were tested for every dose.

Effect of azadirachtin on oviposition activity: In the no- choice trials, *Nerium oleander* leaves were immersed in two concentrations of azadirachtin, 112.5 and 225 mg/ liter, for one minute. The leaves were air-dried and then introduced into cages containing about fifty adults of *S.littoralis* (males and females), provided with a piece of cotton soaked in a 10 % sugar solution, to feed on. In the choice trials, control leaves immersed in water only and treated leaves were distributed in the same cage. The control leaves were also introduced in other seperate cages. The number of egg masses laid in each treatment was recorded.

RESULTS AND DISCUSSION

Effects of feeding second instar stage larvae on azadirachtin –treated leaves: The second instar larvae were the most sensitive to the effects of azadirachtin. As indicated in Table I, the concentration required to cause 50 % reduction of pupation was to 3.67 ppm, while that causing 95 % reduction in adult emergence was 7.8 ppm. The larvae fed on azadirachtin –treated leaves stopped feeding after two 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 in concentrations. The control larvae developed normally to the pupal stage after 9-11 days, while treated larvae were still in the second or third instar based on the concentrations they fed on. To explain this effect, after 9 days. The weight of control larvae was in the overage of 0.41 gm / larva, while that the larvae fed on 10 ppm-treated leaves was in the overage of 0.05 gm / larva. Most of the treated larvae semained inactive for several days (sometimes to 14 days) and then died. The two concentrations 6 and 8 ppm had almost the same effects on the three parameters studied (Table 2). There were no significant differences between the effects of the two concentrations 2 and 4 ppm, except in their effect on pupation.

Table (1): Probit analysis of feeding and contact toxicity of azadirachtin to different larval stages of *S. littoralis*

Parameter	Larval stage	I_{50}* ppm	95 % fiducial limits	I_{95} ppm	95 % fiducial limits	Slope
Pupation	2nd	3.67	3.4-3.96	7.8	7-8.75	4.99
	3rd	10.75	9.84-11.73	30.23	24.8-36.93	3.66
	4th	10.92	8.94-11.69	43.34	32.29-58.8	2.62
	4th**	1.16	1.06-1.26	4.33	3.6-5.46	2.87
Adult emergence	2nd	3.4	3.11-3.72	10.2	8.6-12.17	7.75
	3rd	9.13	8.39-9.94	22.85	19.62-26.66	4.13
	4th	7.62	6.76-8.65	37.77	30.1-47.7	2.37
	4th**	0.55	0.45-0.65	4.25	3.28-6.1	1.86

*I_{50} = the concentration required to cause 50 % reduction in pupation, adult mergence,
**I_{50} = µg / larva (topical application)

Table (2): Effects of feeding 2nd instar larvae of *S. littoralis* on castor bean leaves treated with different concentrations of azadirachtin*

Concentration (ppm)	Pupation %[a]	Adult Emergence %	Larval weight[c]
0.0	88.3 a	71.7 a	0.41 a
1	78.3 b	70 a	0.32 b
2	78.3 b	42.13 b	0.21 c
4	41.7 c	36.7 b	0.18 c
6	13.3d	13.3 c	0.12 d
8	5 d	5 c	0.1 d
10	0.0e	0.0d	0.05e

* Original data were transformed into $\sqrt{percentage}$ before ANOVA and LSD tests.

[a]Values followed by the same letter within a column are not significantly different at the 0.05 level.

[c]Average weight of larva (gm), after 9 days.

Effects on third instar larvae : Third instar larvae were about three times more tolerant to effects of azadirachtin than second instar larvae. The concentrations that inhibited 50 % of pupation and adult emergence were 10.75 and 9.13 ppm, respectively (Table 1). 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. The intensity of symptoms was proportional to concentrations. Egg mass production was severely affected by the treatment. The concentration of 15 ppm almost sterilized completely sterilized the insects (Table 3).

Tabe (3): Effects of feeding 3rd and 4th instar larvae of *S. littoralis* on castorbean leaves treated with different concentrations of azadirachtin*.

Conc	Pupation %ᵃ		Adult emergence %		Egg mass %	
ppm	3rd stage	4th stage	3rd stage	4th stage	3rd stage	4th stage
0.0	93.3 a	94.7 a	92.2 a	88 a	100 a	100 a
2.5	NT	88 ab	NT	74.7 ab	NT	62.3 b
5	84.4 ab	70.6 bc	68.9 b	55.3 bc	41.8 b	11.2 c
10	62.2 bc	60 c	45.5 bc	43.3 cd	17.5 c	9.3 c
15	52.2 c	30 d	20 c	29.3 d	0.9 d	0.0 d
20	0.0 d		0.0 d		0.0 d	
30	NT	6 e	NT	4 e	NT	0.0 d

* Original data were transformed into $\sqrt{percentage}$ before ANOVA and LSD tests.

ᵃValues followed by the same letter within a column are not significantly different at the 0.05 level.

NT = not tested.

Effects on fourth instar larvae:

A: Effects of feeding on azadirachtin-treated leaves: On the basis of I_{50} values, fourth instar larvae were almost as sensitive as third instar ones to effects of azadirachtin. The concentrations that inhibited 50 % of pupation and adult emergence were 10.92 and 7.62 ppm, respectively (Table 1). On the basis of I_{95} percentage of pupation, the fourth instar larvae were more tolerant than those of third instar. The I_{95} values for the two larval instars were 43.34 and 30.23 ppm, respectively. Symptoms of toxicity were similar to those appeared on third instar larvae, but the ratio of malformed pupae and intermediate were higher in case of the fourth instar larvae. The adults with malformed wings or wingless, or with only one pair of wings were noticed. Meisner and Nemny (1992) found that feeding the fourth instar larvae of *S.littoralis* on cotton leaves treated with 20 ppm azadirachtin was effective, which agrees with the present findings. Hashem et al. (1998) reported that feeding the fourth instar larvae of *S. littoralis* on castor bean leaves treated with 0.368 % (3680 ppm) of neem seeds ethanolic extract caused 50 % mortality up to emergence of adults. This distinct high concentration was probably due to the use crude extract, not pure azadirachtin. The topical application of this extract at 10.65g / larva caused 50 % mortality.

B: Contact toxicity (topical application) of azadirachtin: Azadirachtin showed contact toxicity to the fourth instar larvae. The concentrations that caused 50 and 95 % reduction in pupation were 0.55 and 4.25-µg / larva, respectively. These results also agree with those of Meisner and Nemny (1992), who obtained 70 % reduction in pupation with 2-µg / larva. Contact toxicity caused the same symptoms of feeding toxicity. However, contact toxicity of azadirachtin is considered much lower than its toxicity by feeding. Dipping plant leaves in as low concentration as 43 ppm caused 95 % reduction in pupation, while the dose required to aftain the same result by contact was equal to 4.25 µg / larva (4250 ppm). This indicates that azadirachtin is much more toxic by feeding than by contact.

Effects of azadirachtin on oviposition activity: The present results showed that azadirachtin did not prevent females from oviposition on *Nerium oleander* leaves treated with 112.5 and 225 ppm of azadirachtin. These concentrations are more than those used in the field. Many trials proved similar results. Table 4 represents the results of one of these trials. It may be clear that females oviposited in all treatments in both cases of choice and non-choice trials. Moreover, the number of egg masses laid on treated leaves in choice trials, was very close to the number laid on control leaves.

In conclusion, this investigation proved that azadirchtin, formulated as Neemix 4.5, is a successful botanical insecticide. It has been exempted from residue tolerance requirements by the US Environmental Protection Agency for food crop applications (Immaraju, 1998). It could be used in the integrated management programs to control *S.littoralis* to prevent or delay appearance of resistance to conventional pesticides. Some unpublished results showed that after three generations, *S. littoralis* did not develop any considerable degree of tolerance to azadirachtin. Feng and Isman (1995)

Table (4): Number of egg masses laid by *S. Littoralis* on azadirachtin-treaed leaves

Conc (ppm)	Choice trials	No-choice trials
0.0	43	58
112.5	41	NT
225	37	45

NT = not tested

found that after 40 generations, the green peach aphid, *Myzus persicae*, had developed 9- fold resistance to pure azadirachtin, whereas the neem seed extract-treated insects did not. 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 affect. However, it is better to use azadirachtin in sequences with other pesticides.

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REFERENCES

Abbott, W. S.(1925). A method of compairing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265-267.

Chaufaux, J., C. J Muller., C. Buisson, V. Sanchis, D. Lereclus and N. Pasteur (1997). Inheritance of resistance to the *Bacillus thuringiensis* CryIc toxin in *Spodoptera littoralis* (Lepidoptera: Noctuidae). J. Econ. Entomol., 90 (4): 873-878.

El-Sayed, E.I. (1985). Neem (*Azadirachta indica* A. Juss) seeds as antifeedant and ovipositional repellent for the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd). Bull. Entomol. Soc. Egypt. Econ. Ser. Cairo, 13: 49-58.

El-Sayed, E.I. and E.F. Abdalla (1988). Resistance of cotton leafworm, *Spodoptera littoralis* (Boisd) populations, from different governorates of Egypt to some insecticides. Annals of Agric. Sci., Cairo, 33 (2): 1343-1352.

El-Sebae, A.H. (1977). Incidents of local pesticide hazards and their toxicological interpretation. (Proceeding of the UC/ AID, University of Alexandria, A.R.E., Seminar / Workshop in Pesticide Management, 137-157.

Feng, R. and M. B. Isman (1995). Selection for resistance to azadirachtin in the green peach aphid, *Myzus persicae*. Experientia, 51 (8): 831-833.

Finney D.J. (1971) Probit Analysis, 3rd edn, Cambridge University Press, London, 318 p.

Hashem, M; S. M. El-Mesiri,; F. A. El-Meniawi, and I. S. Rawash (1998). Potency of three plant extracts on the developmental stages of the cotton leafworm, *Spodoptera littoralis*, Boisd. Alex J. Agric. Res., 43 (3): 61-79.

Immaraju J. A. (1998). The commercial use of azadirachtin and its integration into viable pest control programs. Pestic Sci., 54: 285-289.

Ishaaya, I.and M. Klein (1990). Response of susceptible laboratory and resistant field stsrains of *Spodoptera littoralis* (Lepidoptera: Noctuidae) to teflubenzuron. J. Econ. Entomol., 83 (1): 59-62.

Issa, Y. H.; M. E. Keddis; M. A. Abdel-Sattar, F.A Ayad and M.A. El-Guindy (1984). Survey of resistance to pyrethroids in field strains of the cotton leafworm, *Spodoptera littoralis* (Boisd) during 1980-1984 cotton growing seasons. Bull. Entomol. Soc. Egypt. Econ. Ser., 14: 405-411.

Keddis, M.A, F.A. Ayad, M.S. Abdel-Fattah and M.A. El-Guindy (1988) .Studies of resistance to urea derivatives and their mixtures with insecticides in field strains of the cotton leafworm, *Spodoptera littoralis* (Boisd), during the cotton seasons 1983, 1984 and 1985. Bull. Entomol. Soc. Egypt. Econ. Ser.,15: 229-234.

Martinez, S.S. and H.F. Van-Emden (2001).Growth disruption, abnormalities and mortality of *Spodoptera littoralis* (Biosuval) (Lepidptera: Noctuidae) caused by azadirachtin. Neotropical-Entomology, 30 (1): 113-125.

Meisner, J. and N. E. Nemny, (1992). The effect of Margosan-O on the development of the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd) (Lep., Noctuidae). J. Appl. Entomol., 113 (4): 330-333.

Salama, S. H., M. S. Foda, and A. Sharaby, (1989). A proposed new biological standard for bioassay of bacterial insecticides vs. *Spodoptera* spp. Tropical Pest Management, 35 (3): 326-330.

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التأثيرات الابادية لمادة الازاديراكتين على حشرة دودة ورق القطن

تــم اختبار المبيد (Neemix 45%) والذى يحتوى ٤,٥% من المادة الفعالة azadirachtin علــى دورة حــياة الاطــوار المختلفة ليرقات دودة ورق القطن *Spodoptera littoralis* عند تغذيتها على ورق خروع معامل بتركيزات مختلفة من مادة الازاديراكتين.

وقد اوضحت النتائج ان الطور اليرقي الثاني هو اكثر الأطوار اليرقية حساسية للمعاملة بهذا المبــيد وكــان التركــيز المتوسط الذى أدى الى خفض معدل الدخول فى طور العذارى لكل من العمــر الــثانى أو الثالــث أو الرابع من اليرقات هما: – ٣,٦٧ ، ١٠,٧٥ ، ١٠,٩٢ جزء فى الملــيون على التوالى. وقد أدت معاملة اليرقات بالازاديراكتين الى عدة تأثيرات تضمنت التوقف عــن التغذية وتثبيط تطور اليرقات الى العذارى كما أدت الى تكون أفراد غير مكتملة النمو من العــذارى وكذا من الحشرات الكاملة. وقد اظهر مركب الازاديراكتين أيضا سمية بالملامسة عند اســتخدامه بجــرعات فوقية على اليرقات ولكن بالمقارنة كانت معدلات الموت وأعراض السمية أعلى عند المعاملة بالمركب عن طريق الغذاء بالفم، وعموما قد أحدثت المعاملة بالمبيد خفضا فى معــدل إنتاج البيض عند التركيزات الأقل من المميتة لليرقات بدرجات متناسبة مع قيمة التركيز وان كــان قــد لوحظ ان لطع البيض يمكن ان تعيش وتفقس فوق أوراق نبات الخروع المعاملة بالمبيد.

والنــتائج بصــفة عامة توضح ان مادة الازاديراكتين يمكن ان تستخدم كمبيد حشرى ضمن برنامج المكافحة المتكاملة بما يساعد على منع او تأجيل ظاهرة مقاومة الحشرة للمبيدات التقليدية المحضرة صناعيا.