EFFECT OF AZADIRACHTIN ON THE CONSUMPTION AND UTILIZATION OF FOOD IN SOME EARLY LARVAL INSTARS OF *SPODOPTERA LITTORALIS* (BOISD.).

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ABSTRACT

The azadirachtin preparation, NeemAzal, was assessed against the Egyptian cotton leafworm Spodoptera littoralis to clarify its possible action on the food metabolism. Six concentration levels were prepared: 1250, 625, 312, 100, 50, and 10 ppm, and given to 2nd instar larvae with the food (Castor leaves). All metabolic parameters were estimated during the 2nd and 4th larval instars. A detrimental effect on the food consumption of 2nd instar larvae was found, irrespective of the NeemAzal concentration level. Almost all estimated metabolic parameters were less than those of control larvae and decreased as the conc. level was increased. Similarly, food cosumption of 4th instar larvae clarified a strong action of NeemAzal.

Keywords: NeemAzal, Food metabolism, *Spodoptera littoralis*.

INTRODUCTION

Over 200 insect species in seven orders are susceptible to neem (Mordue and Blackwell, 1993). Azadirachtin, a tetranortriterpenoid is the major active ingredient in the neem seeds. It causes feeding inhibition and growth disruption in various insect orders, when sprayed on leaves or applied topically (Butterworth and Morgan, 1968, 1971; Rembold and Seiber, 1980). Because of the safety to insect parasitoids, predators and other non-target organisms as compared to the synthetic insecticides, many investigations recommended the use of neem extracts as pest control agents (Joshi *et al.*,1982; Schmutterer and Ascher, 1984; Helpap, 1985; Jayary *et al.*, 1993; Lowery and Isman, 1995; Srivastava *et al.* 1997). NeemAzal is a neem preparation with the azadirachtin (the most active ingredient) content of 20%, and inexpensive production in addition to its safety toxicologically and environmentally (Kleeberg, 1992). Growth and reproductivity of the insect depend on the metabolic efficiencies (Hewitt, 1968; Johnson and Mundel, 1987; Hinks *et al.*, 1991; Lindroth, 1993; Costa *et al.*, 2000). Scriber and Slansky (1981) pointed out that knowledge of these indices are necessary for understanding insect's biology, behaviour and impact in natural ecosystem and agro-ecosystem. The antifeedant activity of Neem on different insect species was detected by many researchers, Rees and Beck, 1976 and Adeyeye and Blum, 1989.

Our objectives in the present study were to investigate the possible effects of this azadirachtin containing preparation, on the food metabolism of the cotton leafworm, *Spodoptera littoralis*.

MATERIALS AND METHODS

A culture of the Egyptian cotton leafworm *Spodopera littoralis* Boisd. was raised at Plant Protection Dept., Faculty of Agriculture, Al-Azhar University according to Ghoneim (1985). Larvae were fed on castor bean leaves (*Ricinus communis* L.) and kept under the laboratory conditions of 27±3 °C and 60-70% RH.

*** NeemAzal assessment.**

The following aqueous concentrations of the tested compound were prepared: 1250, 625, 312, 100, 50, and 10 ppm. Newly ecdysed second instar larvae were fed on clean castor leaves previously dipped once (for 5 minutes) in the required concentrations. Five replicates of treated and untreated (control) larvae (6 larvae/rep.) were carried out for each concentration level. Treated larvae were allowed to feed on treated leaves for 24 h only, then provided with untreated clean plant leaves.

***Metabolic parameters:**

In the present work the following food consumption, absorption and utilization parameters were estimated overall the second and fourth larval instars. Treated and control larvae were weighed before and after feeding, fresh weight was weighed before offering to the larvae, and its fresh weight was recorded after feeding every day. Each replicate of larvae was starved for 3 h before

weighing to ensure an empty intestine. Fresh leaves were kept in rearing jars under the same conditions to estimate the natural loss of moisture, which was used for calculating the corrected weight of the consumed leaves. Weight of faeces is the amount of frass discharged by larvae during the instar. Feeding rate is the amount of food consumed during the feeding period of the instar, generally expressed on a "per day" basis (consumption rate, CR) or on a "per day per unit body mass" basis (relative consumption rate, RCR) (Slansky, 1993). **RCR** = mg consumed food/g mean fresh body weight/day (Slansky and Scriber, 1985). Approximate digestibility (**AD**) = [weight of ingested food-weight of faeces/weight of ingested food] x100. Efficiency of conversion of digested food to body substance. Efficiency of conversion of digested food to body substance (**ECI**) = [weight gain/weight of ingested food] x100. Efficiency of conversion of digested food to body substance (**ECD**) = [weight gain/weight of ingested food-weight of faeces] x100. Growth rate (**GR**) = fresh weight gain during feeding period/feeding period x mean fresh body weight of larvae during the feeding period (Waldbauer, 1968). Relative weight gain (**RWG**) = mg weight gain during the instar/days (Johnson and Mundel, 1987 with correction for a single instar). Assimilation rate (**AR**) = RCR x AD (Scriber and Slansky, 1981). Relative metabolic rate (RMR) was calculated according to Slansky (1993) but corrected for fresh weights as follows: **RMR** = (mg weight ingested food-weight of faeces)/g mean fresh body weight/day. The previous parameters were estimated on the basis of the whole larval instar.

Data obtained were statistically analysed using the student's *t*-distribution refined by Bessel correction (Moroney, 1957) for testing the significance of difference between means.

RESULTS AND DISCUSSION

1) Food Consumption:

On the basis of food eaten overall the second and fourth larval instars of *S. littoralis* (Data given in Table 1), indicate a great reduction of food consumed by both instars as being affected by NeemAzal treatment. This reduction was found to be statistically significant and correlated with the concentration level reversally, irrespective of the affected larval instar. These decrements were proved a sever effect of NeemAzal on 4th instar than do on 2nd one. Data in table (1) showed a remarkable significant reduction in the relative weight gain (RWG), irrespective of the instar, as a result of the reduction in food eaten. Relative consumption rate (RCR) showed the same trend as it decrease significantly by increasing the concentration level, irrespective of the affected instar comparing with the control congeners. Thus, the present results for *S. littoralis* show, generally, an inhibitory action of the tested neem extract on the food consumption. These results agree with that of Linton *et al.*, 1997 who found that Azadirachtin reduced food eaten by *Schistocerca gregaria* at doses of 7 and 5 ug/g-1 body weight and that of Richter *et al.* 1997 who recorded reduced the food consumption in nymphs and adults of *Periplaneta americana using* NeemAzal.

2) Food absorption:

Absorption of food through the gut wall of an insect is commonly indicated by the absorption or digestion efficiency under the usual term "approximate digestibility "(AD). It estimates the percentage of ingested food that is digested and assimilated (Slansky and Scriber, 1985). Depending on the data presented in Table (2), the AD values of 2nd instar larvae increased, but in no certain trend. Its change ranged from +0.7% (at 1250 ppm) to −5.2 % (at 50 ppm). Its values decreased during the 4th larval instar only at the two middle concentration levels but increased at other ones. Recalling the data of table (1), and comparing the food consumed it is clear that the larvae compensate the decrease in food intake by increasing the food digestibility, regardless of the affected instar. This result agrees with that obtained by Radwan *et al.* (1986) for *S. littoralis* by the action of diflubenzuron and triflumuron throughout the larval period from 4th instar to 6th instar. Also, our results of AD values agree with those obtained by Ghoneim (1994) for the last nymphal instar of *S. gregaria* by the action of pyriproyfen. And disagrees with that obtained by Amr (1986) after using garllic acid against the spiny bollworm *Earias insulana*, Mansour (1981) for *S. littoralis*; Farag (1991) for the same species and Ismail (1995) after using fenoxycarb against *S. gregaria*.

Table (I): Food consumption (mg±SD) of *Spodoptera littoralis* as affected by NeemAzal treatment of second instar larvae.

Conc. levels (ppm)	Second larval instar			
	Food consumed	RWG	RCR	Change %
1250	4.3±0.95d	1.92±0.40d	0.43±0.05d	-67
625	5.3±0.6d	2.3±0.53d	0.53±0.04d	-59
312	6.0±1.00d	2.84±0.27d	0.56±0.05d	-57
100	6.3±0.69d	3.10±0.21d	0.58±0.06d	-55
50	7.7±1.15d	3.70±0.56d	0.70±0.16d	-46
10	10.3±1.53c	4.54±0.61c	0.88±0.15c	-32
Control	16.0±1.00c	6.40±0.78a	1.30±0.16a	--
	Fourth larval instar			
1250	--	--	--	--
625	32.33± 6.42d	3.9±0.57d	0.68±0.05c	-26.88
312	72.0± 1.00d	4.66 ± 1.40d	1.33±0.33c	+43.01
100	125.0±16.40a	7.90±0.90d	1.27±0.05b	+36.56
50	142.3±7.77c	9.71 ± 1.78d	1.28±0.15b	+37.63
10	245.0±14.73b	18.1± 1.10c	1.11±0.11a	+19.35
Control	277.2±10.30a	24.38 ± 4.87a	0.93±0.03a	--

RWG: relative weight gain, RCR: relative consumption rate of food, . Means ± SD followed with the same letter (a) are not significantly different (P> 0.05), (b): significantly different (P<0.05), (c): highly significantly different (P<0.01) (d): very highly significantly different (P<0.001). ---: no resulted larvae

Table (II): Food aproximate digestibility (mean mg ± SD) of *Spodoptera littoralis* larvae as affected by NeemAzal.

Conc. (ppm)	2nd larval instar		4th larval instar	
	AD	change%	AD	change%
1250	70.0±2.2a	-0.7	73.08±1.9a	+4.41
625	72.3±2.3a	+3.0	76.76±7.1b	+9.67
312	73.3±1.9a	+4.0	68.36±2.3a	-4.36
100	71.1±2.5a	+0.9	67.99±3.6a	-3.74
50	74.2±3.1a	+5.2	70.02±3.4a	+3.99
10	71.0±3.7a	+0.7	72.71±3.7a	-0.69
Control	70.5±1.3a	--	69.99±2.7a	--

AD: aproximate digestability; Conc., a, b, c, d: see footnote of Table (1).

3)Food Utilization:

After absorption of the digested food, some of the metabolites are used for metabolic energy and a certain portion is converted to the biomass. ECI is a measure of the insect's ability to utilize food for growth.. The second indicator of food utilization is the efficiency of conversion of digested food (ECD) which is sometimes called " Net Growth Efficiency" (or "Metabolic Efficiency") It estimates the percentage of assimilated food to biomass (Slansky and Scriber, 1985).

A dramatic impact of NeemAzal on ECI and ECD was observed for both 2nd and 4th instar larvae at some concentration levels table (3). NeemAzal exerted an inhibitory action on AR of 2nd instar larvae which decreased by increasing concentration (Table 4). The second instar larvae, treated with NeemAzal have the ability to convert the ingested or digested food to biomass as ECI did not affect by this treatment, except in the case of the highest concentration level. In contrast, the latent effect on 4th instar larvae remarkably impaired the capabilities of them to do such. In spite of this the effect on the fourth larval instar has no certain trend, when the larvae died at the highest concentration level, 1250ppm and increased their their ability to utilize food for growth and in turn their metabolic efficiency at the concentration level, 650ppm. In addition, NeemAzal exhibited an inhibitory action on ECD of both instar larvae especially at the highest concentration levels and

extend this effect to the 4th instar larvae clearly. Generally, NeemAzal prohibited partially the ability of these early instars to convert the ingested or digested food to their biomass. This inhibitory action on both parameters was estimated and tabulated as change percentage to certify this negative effect as mentioned before. Similarly, different IGRs reduced ECI and ECD of various insect species such as diflubenzuron and triflumuron against *S. littoralis* (Radwan *et al.*, 1986), fenarimol against *S. littoralis* (Farag, 1991), fenoxycarb against *S. gregaria* (Ismail, 1995), tebufenozide against *S. littoralis* (Bream *et al*, 1999).

4) Relationship between food metabolic and somatic growth:

The assimilation rate (AR) and relative metabolic rate (RMR) are metabolic parameters presented in Table 4 to shed some light on a detectable metabolic action of NeemAzal. AR indicates the ability of larvae to assimilate the digested and absorbed food overall the instar. It is obvious that NeemAzal exerted an inhibitory action on AR of 2nd instar larvae which decreased by increasing conc. The metabolic effect of NeemAzal reflected on the RWG and GR, which had been drastically reduced. On the other hand, NeemAzal

Table (III): Food utilization of *Spodoptera littoralis* as affected by NeemAzal treatment of second instar larvae.

Instars / Conc. (ppm)	ECI	Change%	ECD	Change%
	Second larval instar			
1250	67.4±5.1b	-17	90.3±7.2d	-22
625	77.4±3.7a	-5.0	105.1±8.4b	-9.0
312	78.3±4.1a	-4.0	104.4±7.3b	-11
100	79.4±2.7a	-2.0	107.1±9.3a	-8.0
50	79.4±3.7a	-3.0	107.2±8.7a	-8.0
10	77.7±2.5a	-4.0	107.0±6.3a	-8.0
Control	81.3±3.1a	--	116.1±7.9a	--
	Fourth larval instar			
1250	---	---	---	---
625	42.15±3.11d	+36.85	60.32±6.1d	+32.57
312	22.62±6.55c	-26.56	32.79±9.4c	-27.9
100	22.45±1.61c	-27.11	33.84±1.4c	-25.63
50	23.08±2.9b	-25.06	35.13±4.39c	-22.79
10	25.95±2.9b	-15.75	37.82±4.52c	-16.88
Control	30.8±1.02a	--	45.5±0.58a	--

ECI: Efficiency of conversion of ingested food, ECD: Efficiency of onversion of digested food, a, b, c, d : see the footnote of table (1).

Table (IV): The correlation of GR to AR and RMR of *Spodoptera littoralis* as affected by NeemAzal treatment of second instar larvae.

Instars / Conc. (ppm)	Second larval instar			Fourth larval instar		
	GR	AR	RMR	GR	AR	RMR
1250	0.15±0.01d	13.1±0.77d	1.57±0.13c	--(a)	--	--
625	0.19±0.01d	17.5±1.1d	1.65±0.17b	6.2±0.1d	47.64±4.30d	--
312	0.22±0.01d	20.1±1.7d	1.74±0.12a	6.0±0.2d	91.45±22.48d	1.20±0.1
100	0.22±0.01d	21.3±1.2d	1.74±0.13a	8.0±0.1d	84.01±4.08d	2.36±0.03
50	0.27±0.02d	24.4±1.6d	1.76±0.13a	9.0±0.1c	84.25±10.4d	3.10±0.1
10	0.35±0.03c	31.5±1.5d	1.84±0.18a	13.0±0.5b	76.23±8.30b	4.20±0.4
Control	0.54±0.03a	47.5±2.5a	1.86±2.0a	15.0±0.5a	63.25±0.69a	3.95±0.09

GR: Growth rate (X100), AR: Assimilation rate (X100), RMR: Relative metabolic rate (X100). a, b, c d, (a): see the footnote of table (1).

exhibited a different effect on AR, RMR, and GR of the 4th instar larvae. It promoted these larvae to attain higher AR, especially at the higher four conc. levels (10,50,100 and 312). However, NeemAzal had considerably influenced the AR and RMR of 4th instar larvae as it does on RWG and GR. Data provided in table (4) reveal the highly significantly declined rate of food assimilation throughout the second larval instar as compared to the control congeners and the 4th larval instar. The inhibitory action of this formulation on food metabolism can be, also, observed by RMR, which was depressed after treating larvae with higher two concentration levels. AR of 2nd instar larvae was highly significantly declined than RMR. With few exceptions, the two metabolic rates, AR and RMR were positively correlated to the relative consumption rate and RCR of the food because all these parameters were subjected to an inhibitory action of NeemAzal (Tables 1 and 3). Thus, our determinative results, indicate highly drastic inhibitory action of NeemAzal on AR and RMR and this reflected on the growth rate (GR), irrespective of the instar investigated (table 4) indicating a prolonged influence of the tested compound. These effects may be due to the inability to metamorphose properly, which is possibly based on the disturbance of the hormonal regulation. Similar conclusion was obtained by Beck and Reese, (1976) on *A. ipsilon*; Dahlman, (1977) on *Manduca sexta*, Sundaramurthy, (1977) on *S. litura;* Radwan *et al.*, (1986) on *S. littoralis*; (Farag, 1991), on *S. littoralis* and Bream *et al.*, (1999). In addition, several authors explained the depressed GR or depleted RWG by the decreasing amounts of food consumed. This interpretation may be also suggested in the present study because the data of tables 1-3 clearly show detrimentally reduced RWG and food consumed by both instar larvae studied.

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الملخص العربي

الاستهلاك وتغيرات أيض الغذاء في يرقات *سبودوبترا ليتوراليس*

(حرشفيات الأجنحة : الليليات)

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استخدم في هذه الدراسة ستة تركيزات من المستخلص النباتي المعروف باسم النيمازال (٢٠ %) وهى :

١٢٥٠ ، ٦٢٥ ، ٣١٢ ، ١٠٠ ، ٥٠ ، ١٠ جـزء من المليون، ومعاملة يرقات الدور الثاني لدودة ورق القطن سبودوبترا ليتوراليس بطريقة التغذية لمدة ٢٤ ساعة فقط.

تـم تقديـر كل المعايير الأيضية خلال الدورين الثاني والرابع. وقد لوحظ تأثير ضار على استهلاك الغذاء خلال الدور الثاني بغض النظر عن تركيز النيمازال.

وجـد أن كـل المعايير الأيضية كانت أقل من هذه المسجلة للمجموعة الضابطة وتقل مع زيادة التركيز، وكان استهلاك الغذاء خلال الدور الرابع متأثرا بالنيمازال على نحو مشابه لما حدث خلال الدور الثاني.