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Neemazal Effects on the Consumption and Utilization of Food in Some Early Larval Instars of the Cotton Leafworm, *Spodoptera littoralis* Boisd. (Noctuidae:Lepidoptera)

H.A. Mohamed, [1]K.S. Ghoneim and [1]A.S. Bream
Faculty of Agriculture, [1]Faculty of Science, Al-Azher University, Madenit Nasr, Cairo, Egypt

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 intake and consumption of 2nd instar larvae was found, irrespective of the Neemazal conc. level. All estimated metabolic parameters were less than those of control larvae and decreased as the conc. level was increased. Similarly, food intake and faeces output of 4th instar larvae had been undergone to a strong action of Neemazal. The Approximate digestibility (AD) values of 2nd instar larvae increased, but in no certain trend. Its changes ranged from + 0.7 5 (at 1250 ppm) to +5.2% (at 50 ppm). Its values decreased during the 4th larval instar only at the two middle conc. levels but increased at other ones. A dramatic impact of Neemazal on Efficiency of conversion of ingested food to the body substance (ECI) and Efficiency of conversion of digested food to the body substance (ECD) was observed for both 2nd and 4th instar larvae at some conc. levels. Neemazal exerted an inhibitory action on Assimilation rate (AR) of 2nd instar larvae which decreased by increasing conc. The metabolic effect of Neemazal reflected on Relative weight gain (RWG) and Growth rate (GR) which had been drastically reduced. On the other hand, Neemazal exhibited a different effect on AR, Relative metabolic rate (RMR), RWG and GR of 4th instar larvae. It promoted these larvae to attain higher AR, especially at the higher four conc. levels. However, AR or RMR of 4th instar larvae had not been considerably influenced as RWG and GR by Neemazal.

Key words: Neemazal, consumption, utilization, growth, weight gain, metabolism, absorption, *Spodoptera littoralis*

Introduction

Antifeeding properties of different plant extracts as control measures of several phytophagous pests has attracted the attention of many researchers all over the world (Wright, 1970; Khader *et al*., 1986). Azadirachtin, a neem-seed extract, has antifeedant activity against the desert locust *Schistocerca gregaria* (Pradhan *et al*., 1963; Zanno *et al*., 1975). Several studies that followed on, azadirachtin confirmed its strong feeding inhibitory action on a number of insect species (Ladd *et al*., 1978; Warthen, 1979; Guerrini, 2000). Disruption of growth after azadirachtin application was believed to be due to feeding inhibition but it was shown that growth disruption could occur without inhibition of feeding in Holometabola. Neemazal (a commercial neem preparation with an azadirachtin content of 20%) was proven to be a growth retardant (Zuber and Bollhalder, 1997; Ghoneim *et al*., 2000; Mohamed *et al*., 2000) and phagodeterrent (Richter *et al*., 1997). Objective of the present work was to determine the effects of Neemazal on food consumption, absorption and utilization in a purpose to disclose the role of azadirachtin (active material in the Neemazal) in food metabolism of the early larval instars of *Spodoptera littoralis*.

Materials and Methods

A culture of the Egyptian cotton leafworm *Spodoptera Littoralis* Boisd. had been started by a sample of pupae obtained from the permanent culture of susceptible strain of Lab. of Insecticides, Agric. Res. Center, Doqqi, Giza. The maintenance of the culture was carried out according to Ghoneim (1985). Larvae were provided with castor-oil leaves (*Ricinus communis* L.) as a fresh food. All experimental and culture vials were kept at 27±3°C and 60-70 % RH.

Neemazal, as an emulsifiable concentrate, was used in a range of concentration levels of: 1250, 625, 312, 100, 50 & 10 ppm. Newly moulted 2nd instar larvae were allowed to feed Neemazal-treated leaves for 24h only and then have been fed with untreated leaves. Six replicates of larvae (5 larvae/rep) were used for each concentration level. Control larvae were provided with clean untreated leaves.

The nutritional parameters were estimated for both second and fourth larval instars.

Treated and control larvae were weighed before and after feeding, fresh weight of food was recorded before and after be given to larvae. Fresh leaves were kept in Petri dishes without larvae under the same conditions to estimate the natural loss of moisture for calculating the corrected weight of the ingested food. Faeces discharged by larvae were weighed. Feeding rate was 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] X 100. Efficiency of conversion of ingested food to body substance (ECI) =[weight gain/weight of ingested food] X 100. Efficiency of conversion of digested food to body substance (ECD) = [weight gain / weight of ingested food – weight of faeces] X 100. Growth rate (GR) = fresh weight gain during the 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 weight as follows: RMR = (mg weight of ingested food – weight of faeces) /g mean fresh body weight / day. These studies may help to clear the metabolic efficiencies which can affect growth (Hewitt, 1968; Johnson and Mundel, 1987; Hinks *et al.*, 1991).

Date obtained were statistically analyzed using the Student's *t*- distribution and refined by Bessel correction (Moroney, 1956) for testing the significance of difference between means.

Results

Data of Table 1 show a detrimental effect on the food intake and consumption of 2nd instar larvae, irrespective of the Neemazal concentration-level. The calculated values of all parameters were less than those of control larvae and decreased gradually as the Neemazal conc. level was increased. For some details, 1250 ppm- treated larvae ate ¼ of food eaten by their control congeners (4.3 ± 0.95 vs. 16.0 ±1.00 mg) while 10 ppm- treated larvae ate 2/3 of food eaten by controls (10.3±1.52 vs. 16.0 ±1.00 mg). Whereas 1250 ppm – treated larvae discharged ¼ faecal amount of controls (1.3±0.16 vs. 4.8±0.20 mg), 10 ppm-treated larvae discharged 3/5 faecal amount of controls (2.9±0.22 vs. 4.8 ±0.20 mg).

In respect to the relative consumption rate (RCR), a gradual retardation was observed in the Neemazal-treated larvae reaching 1/3 RCR of the control larvae, at the highest conc. level at which the change % was twice of it at the lowest conc. level.

Also, those 4th instar larvae produced from the treated 2nd instar ones had been studied. It is quite clear from Table 2 that a strong action of Neemazal on both food intake and faeces output had been exerted. These two parameters had been reduced in a dose- dependent course. As for example, the highest conc.- treated larvae ate a half of that amount eaten by their control congeners (125.3±5.9 vs. 233.2±16.4 mg) and produced faeces as a half of that produced by controls (69.9±2.7 vs. 147.3±9.3 mg).

Also, the available results clarified the dramatic impact of the present neem extract on RCR, but in no certain trend, because the two lower conc. levels resulted in reduced RCR (estimated as –10.9 and – 5.9% at 50 and 10 ppm, respectively) while the higher conc. levels led to increased RCR of which change % ranged from + 31.9% (at 100 ppm) and + 41.5% (at 1250 ppm).

Table 3 contains the data indicating a positive effect of Neemazal on the approximate digestibility (AD) of 2nd instar larvae because it increased, but in no certain trend. The change ranged from + 0.7% (at 1250 ppm) to +5.2% (at 50 ppm). In addition, the 4th instar larvae had variable values of AD since change decreased only at the two middle conc. levels (-2.2 and –2.9 % at 312 and 100 ppm, respectively). Neemazal at other conc. levels (lower or higher) enhanced those larvae to achieve higher AD than that of control larvae since the change ranged from + 0.1% (at 50 ppm) to + 9.8 % (at 625 ppm). Shortly, Neemazal exhibited variable effects on AD depending on the conc. level, because it exerted an inhibitory action at certain coc. levels and stimulatory action at the other ones.

Arguing the data of Table 4, suppressed efficiency of 2nd instar larvae to convert both ingested (ECI) digested (ECD) food into their biomass by the action of Neemazal has been clearly seen. The strongest effect on these utilization parameters was obtained at the highest conc. level (67.4 ± 5.1 vs 81.3 ± 3.1 ECI of control larvae and 90.3 ±7.2 vs 107.0 ± 7.9 ECD of control larvae). Also, ECI change increased parallely with the increasing conc. to be ranged from – 1.9% (at 10 ppm) to –17.1% (at 1250 ppm). In addition, ECD change ranged from – 7.8% (at 10 ppm) to –22.2% (at 1250 ppm). It is noteworthy to mention that, Neemazal exhibited an effect on ECI and ECD of 4th instar larvae similar to its effect on 2nd instar larvae. With an

Table 1: Food consumption (mg ± SD) of 2nd instar larvae as affected by Neemazal treatment of *Spodoptera littoralis*

Conc. levels (ppm)	Food intake	Faeces output	RCR	change %
1250.0	4.3±0.95***	1.3±0.16***	0.43±0.05***	-66.6
625.0	5.3±0.60***	1.5±0.25***	0.53±0.04***	-59.2
312.5	6.0±1.00**	1.6±0.18***	0.56±0.05***	-56.9
100.0	6.3± 0.69***	1.8±0.18***	0.58±0.06***	-55.4
50.0	7.7±1.53***	2.0±0.16***	0.70±0.16***	-46.2
10.0	10.3±1.53***	2.9±0.22***	0.88±0.15***	-32.3
Control	16.0±1.0	4.8±0.20	1.30±0.16	---

Conc. levels: concentration levels. RCR: relative consumption rate of food. NS: not significant, (P>0.05). *: significantly different (P>0.05), **: highly significant (P<0.01) ***: very highly significant. (P<0.001).

Table 2: Food consumption (mg ± SD) of 4th instar larvae as affected by Neemazal treatment of *Spodoptera littoralis*

Conc. levels (ppm)	Food intake	Faeces output	RCR	change %
12500.0	125.3±5.9***	69.9±2.7***	1.91±0.23***	+41.5
625.0	148.7±4.7***	76.1±1.3***	1.95±0.11***	+44.4
312.5	153.0±6.1***	85.9±5.5***	2.00±0.21***	+48.2
100.0	168.7± 8.4***	108.4±6.9***	1.78±0.08***	+31.9
50.0	182.3±4.7 ***	117.8±6.0***	1.21 ±0.04***	-10.9
10.0	206.3±7.4**	123.9±8.5 **	1.27±0.11 NS	-5.9
Control	233.2±16.4	147.3±9.3	1.35±0.06	---

Conc. level, RCR, ---, NS, * , **, ***: see the footnote of Table (1).

Table 3: Food absorption and utilization of 2nd instar larvae as affected by Neemazal treatment of *Spodoptera littoralis*

Conc. levels (ppm)	AD	Change%	ECI	Change%	ECD	Change%
1250.0	71.0±2.2NS	+0.7	67.4±5.1***	-17.1	90.3±7.2***	-22.2
625.0	72.3±2.3 *	+2.6	77.4±3.7 NS	-4.8	104.1±7.4 *	-10.3
312.5	73.3±1.9 *	+40	78.3±4.1 NS	-3.7	105.4±8.3NS	-9.2
100.0	71.1±2.5NS	+0.9	79.4±2.7 NS	-2.3	107.1±9.3NS	-7.8
50.0	74.2±3.1 *	+5.2	79.4±3.7 NS	-2.3	107.2±8.7NS	-7.8
10.0	69.0±3.6NS	0.7	79.7±3.5 NS	-1.9	107.0±6.3NS	-7.8
Control	70.5±1.3	---	81.3±3.1	---	116.1±7.9	---

Conc. levels, RCR, ---, NS, * , **, ***: see the footnote of Table (1). AD: Approximate digestibility, ECI: Efficiency of conversion of ingested food, ECD: Efficiency of conversion of digested food.

Table 4: Food absorption and utilization of 4th instar larvae as affected by Neemazal treatment of *Spodoptera littoralis*

Conc. levels (ppm)	AD	Change%	ECI	Change%	ECD	Change%
1250..0	73.1±1.9	+4.6	14.3±1.7***	-42.3	19.2±1.19***	-45.9
625.0	76.8±7.1*	+9.8	14.6±0.8***	-41.1	19.6±1.90***	-44.8
312.5	68.4±2.5NS	-2.2	14.5±1.6***	-41.5	21.3±2.86***	-40.0
100.0	67.9±3.6NS	-2.9	16.4± 0.8***	-33.9	24.2±2.05***	-31.8
50.0	70.0±3.4NS	+0.1	25.8±1.0 NS	+4.0	36.9±2.90NS	+3.9
10.0	72.7±3.7NS	+4.0	24.6±2.3 NS	-0.8	33.9±3.35NS	-4.5
Control	69.9±2.7	---	24.8±1.3	---	35.5±0.27	---

Conc. levels, RCR, ---, NS, * , **, ***: see the footnote of Table (1). AD:, ECI:, ECD: see the footnote of Table (3).

Table 5: The Correlation of AR and RMR to RWG and GR of 2nd instar larvae as affected by Neemazal treatment of *Spodoptera littoralis*

Conc. levels (ppm)	AR	RMR	RWG	GR
1250.0	13.1±0.77***	1.57±0.13NS	1.9±0.40***	15.3±1.21***
625.0	17.5±1.10***	1.65±0.17NS	2.3±0.53***	19.2±1.32***
312.5	20.1±1.77***	1.74±0.12NS	2.8±0.27***	22.4±1.53***
100.0	21.3±1.20***	1.74±0.13NS	3.1±0.21***	22.3±1.60***
50.0	24.4±1.62***	1.76±0.13NS	3.7±0.56***	27.1±2.50***
10.0	31.51±1.53***	1.84±0.18NS	4.5±0.61***	35.4±3.22***
control	47.5±2.50	1.86±2.00	6.4±0.78	54.4±3.16

Conc. levels, RCR, ---, NS, * , **, ***: see the footnote of Table (1). AR: Assimilation rate (x 100), RMR: Relative metabolic rate (x 100), RWG: Relative weight gain, GR: Growth rate (x 100)

Table 6: The Correlation of AR and RMR to RWG and GR of 4th instar larvae as affected by Neemazal treatment of *Spodoptera littoralis*

Conc. levels (ppm)	AR	RMR	RWG	GR
1250.0	13.8±1.41***	2.8±0.13NS	4.9±0.71***	4.0±0.71***
625.0	14.9±0.90***	3.3±0.12***	6.4±0.43***	4.9±0.10***
312.5	13.7±1.81***	2.9±0.13NS	6.5±0.92***	5.2±0.63***
100.0	12.1±1.02***	2.9±0.06NS	8.1±0.73***	6.0±0.44***
50.0	8.5±0.65NS	2.7±0.13NS	14.6±0.82***	9.0±0.39**
10.0	8.4±0.99NS	3.0±0.18*	15.7±0.95**	9.8±0.46**
control	9.4±0.77	2.7±0.19	19.0±1.66	11.0±0.60

Conc. levels, RCR, ---, NS, * , **, ***: see the footnote of Table (1). AR: , RMR:, RWG:, GR: see the footnote of Table (5) exception, reduced ECI and ECD consecutively related to Neemazal conc level. The ECI change ranged from – 0.8% (at 10 ppm) to –42.3% (at 1250 ppm) and ECD change ranged from – 4.2 to – 45.9%, at these conc. levels.

Depending on the data of Table 5, it is quite clear that the Neemazal exerted an inhibitory action of the assimilation rate (AR) of 2nd instar larvae, which decreased by ascending conc. Control AD was found as 3.6 times of AR of larvae treated with the highest conc. and as 1.5 times of it at the lowest conc. Also, the same table shows suppressed, but not statistically significant, relative metabolic rate (RMR). This metabolic effect of Neemazal reflected on the relative body weight gain (RWG) and growth rate (GR) which had been drastically reduced. The control larvae obtained RWG of 3.4 times higher than that of 1250 ppm-treated larvae and of 1.4 times higher than that of 10 ppm-treated larvae. A similar case was of control GR which estimated in 3.6 times higher than that of 1250 ppm-treated larvae and in 1.5 times higher than of 10 ppm-treated larvae.

In connection with the 4th instar larvae, Neemazal exhibited a different effect on these metabolic and somatic parameters. According to the data distributed in Table 6, Neemazal promoted the 4th instar larvae to attain AR higher than that of control congeners, especially at the higher four conc. levels. Reversely, the lower two conc. levels resulted in depressed AR. Moreover, Neemazal induced these larvae to achieve higher RMR than that controls, or at least similar to it (2.7 ± 0.13 at 50 ppm vs. 2.7± 0.19 of controls).

Each of RWG and GR had been considerably affected by Neemazal consecutively depending on the conc. level. As for example, RWG was calculated as 4.9 ± 0.71mg (at the highest conc. Level) and 15.7 ± 0.95 mg (at the lowest one) vs. 19.0 ± 1.66 mg of controls. In other words, RWG of control larvae became 3.9 times more than that of larvae treated with the highest conc. and 1.2 time more than that of larvae treated with the lowest concentration.

On the other hand, Neemazal reducing effect on GR increased as the conc. was increased and the calculated GR was found as 1/3 GR of control correspondings (at the highest conc. level) and 4/5 GR of those controls (at the lowest conc. level). In the light of these data, it was easily concluded AR or RMR had not been influenced as RWG and GR by the action of Neemazal.

Discussion

Food ingestion and consumption: Reduced food ingestion and faecal production had been estimated for different insect species by the action of various insect growth regulators (IGRs) and plant extracts (Sundaramurthy, 1977; Farag, 1991; Ghoneim, 1994; Jagannadh and Nair , 1997; Linton *et al.*, 1997; Richter *et al.*, 1997; Bream *et al.*, 1999). A detrimental effect on the food intake and consumption of 2nd instar larvae was found in the present study on *Spodoptera littoralis*, irrespective of the Neemazal concentration level. All estimated metabolic parameters were less than those of control larvae and decreased as the concentration. was increased. Similarly, food intake and faeces output of 4th instar larvae had been subjected to a strong action of Neemazal. These results agreed, to a great extent, with those results obtained by several authors for various species (e.g. Huang *et al.*, 2000 ; Hussien, 2000; Guerrini, 2000). Also, significant inhibition of food intake in 5th instar nymphs of *S. gregaria* was achieved by the peptide cholecystokinin, neuropeptide Y, galanin and bombesin (Wei *et al.*, 2000).

On the contrary, Abid *et al.* (1978) observed an enhancement of food consumption for different insect species by some IGRs. Also, high dose levels of pyriproxfen induced the food ingestion and consumption in *S. gregaria* (Ghoneim, 1994).

Food absorption and utilization: The approximate digestibility (AD) commonly indicates the absorption of food through the gut wall of an insect (Slansky and Scriber, 1993). Decreased AD was determined by Amr (1986) in *Earias insulana*, Farag (1991) in *S. littorallis*, Ismail (1995) in *S. gregaria*, by different IGRs. Dissimilarly, AD values of 2nd instar larvae of *S. littoralis*, in the present study, increased, but in no certain trend . Its change ranged from +0.7% (at 1250 ppm) to +5.2% (at 50 ppm). On the other hand, AD decreased during the 4th larval instar only at 312 and 100 ppm but increased at other concentration levels. However, significantly increased AD was determined by some author (Meisner *et al.*, 1982; Antonious and Hegasy, 1987; Gonzalez *et al.*, 1992; Abou El - Ghar *et al.*, 1996; Hussein, 2000; Garside *et al.*, 2000). However, increased AD value is suggestive of the attempts made by the insect to compensate for reduced consumption and utilization of food in order to maintain growth rates (Reese and Beck, 1976).

The most important measures of the food utilization are: efficiency of conversion of ingested food (ECI) and of digested food (ECD) into the biomass. Different IGRs reduced the food utilization (in suppressed ECI and ECD) of various insect species as an effect of diflubenzuron and triflumuron on *S. littoralis* (Radwan *et al*, 1986), fenarimol on the same species (Farag, 1991), fenoxycarb on *S. gregaria* (Ismail, 1995) and tebufenozide on *S. littoralis* (Bream *et al.*, 1999). In accordance with these results, a dramatic impact of Neemazal on these metabolic efficiencies were estimated for both 2nd and 4th instar larvae of *S. littoralis* in the present study. AZT (the active material in Neemazal) causes primary antifeedant effects through its detection by mouth-part chemoreceptors (Haskell and Mordue, 1969). Distinct from this is the secondary effect of AZT on normal gut function which results in a flaccid semi-full gut, a reduction in the efficiency of protein digestion and a suppressed level of feeding (Mordue *et al*., 1986; Timmins and Reynolds, 1992; Nasiruddin and Mordue, 1994). On the other hand, peritrophic membrane is an extracellular chitin material lining the midgut and has a very important role in the food digestion and absorption (Clarke *et al*., 1977). In the present study, the detrimental effect of AZT (in Neemazal) on the food absorption and utilization might be due to its effect on this membrane (Reynolds, 1987; Jagannadh and Nair, 1997).

Reflection of metabolic effects on somatic growth: Neemazal, in the present study, exerted an inhibitory action on the assimilation rate (AR) of 2nd instar larvae which decreased by increasing conc. The metabolic effect of Neemazal reflected on the relative weight gain (RWG) and growth rate (GR) which had been drastically reduced. On the other hand, Neemazal exhibited a different effect on AR, RMR, RWG and GR of 4th instar larvae. It promoted these larvae to attain higher AR, especially at the higher four concentration levels (1250,625, 312 and 100 ppm). However, AR or RMR of 4th instar larvae had not been considerably influenced as RWG or GR by Neemazal. Various authors an observed exiguous dependence of RWC and GR on AR and RMR in several species by some IGRs and plant extracts (Beck and Reese, 1976; Reese and Beck, 1976; *Agrotis ipsilon*, Dahlam, 1977; in *Manduca sexta*; Sundaramurthy, 1977; in *Spodoptera litura*; Radwan *et al*., 1986; Farag, 1991 and Bream *et al*., 1999; in *S. littoralis*; Ghoneim, 1994 and Ismail, 1995; in *S. gregaria*). Such dependence and affected food consumption and utilization confirms the action of Neemazal as a growth retardant and a phagodeterrent which was also concluded in some insects (Richter *et al*., 1997).

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