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Effect of *Melia azedarach* extract on larval development and reproduction parameters of *Spodoptera littoralis* (Boisd.) and *Agrotis ipsilon* (Hufn.) (Lep. Noctuidae)[1)]

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Abstract

Methanolic extract of *Melia azedarach* fruits enriched by washing with petrolether and ethyl acetate was used for laboratory treatments of two lepidopteran pests in Egypt. The experiments were carried out with concentrations of 10, 15, 25, 50, 100 and 1000 ppm in a diet and compared with control insects. In both insects food consumption, weight gain and conversion of ingested food (ECI) in body matter decreased with increasing extract amounts. The conversion of digested food (ECD) was lowered gradually by using higher concentrations of *Melia* extract. Some antifeedant activity was observed in larvae of *S. littoralis* and *A. ipsilon*. The percentage of mortality increased with application of higher concentrations of *Melia* extract in both species. Starting from 3rd larval instar the larvae of both species reduced significantly their weight until pupation in 25 ppm and higher extract concentrations, while the larval period was prolonged. The pupal weight was significantly reduced at 15, 25 and 50 ppm. At higher concentrations the larvae failed to pupate. Duration of pupal period was affected only in *A. ipsilon*. All reproduction parameters, as period of oviposition, fecundity, fertility and longevity of males and females were affected using emerged adults from treated larvae with concentrations of 10, 15 and 25 ppm *Melia* extract. In *S. littoralis* no adult emerged from pupae originated from larvae treated with 50 ppm and higher amounts and no larva hatched from eggs laid by adults treated with 25 ppm *Melia* concentration as larvae. In both species the oviposition period was shortened at 15 and 25 ppm extract, the fecundity and fertility were drastically reduced, and the longevity of males and females was reduced. Cross sections of the midgut showed that the epithelial cells are destroyed in both pests. This can be one of the reasons for the observed effects.

1 Introduction

During the last 5 decades the application of the various groups of synthetic pesticides, like chlorinated hydrocarbons, organophosphates, carbamates and also pyrethroids established some key problems in plant protection as the development of resistance against the insecticide applied, destroying the environment by killing many non target organisms, long storage in chlorinated hydrocarbons, sometimes very slow degeneration and in many cases high toxicity to men and domestic animals (SCHMIDT, 1986). The application of plant extracts as allelochemicals can be a possible alternative for controlling pest insects in many cases. Plant extracts with insecticidal effects are metabolized easily and do not burden the environment. In most cases they are harmless for men, mammals and birds and more specific against the target insects (KNÜSLI, 1977). Almost only plant feeding insects are affected, and their natural enemies are protected.

In this context plants belonging to Meliaceae family are most important because of the ingredients belonging to triterpenoids and limonoids (KRAUS et al., 1985, 1987, SRIVASTAVA and GUPTA, 1985; KRAUS, 1986; YAMASAKI et al., 1988). The extracts of different parts of the plants have various effects on the insects, e. g. antifeedant, growth disruption, prolongation of larval development, disturbances in metamorphosis and morphogenetics (JACOBSON, 1986).

The most important plant is the Neem tree, *Azadirachta indica* A. Juss (SCHMUTTERER, 1995).

This species is native to the tropical and some parts of the subtropical belt of the earth. This means, it is distributed mainly in developing countries and for the farmers it is easy and not expensive to prepare extracts with insecticidal activity (JOSHI, 1987). Many investigations are carried out in isolation, identification and screening to find out the active principle (SCHMUTTERER al., 1991; SCHMUTTERER and ASCHER, 1984, 1987). The most important ingredients of the Neem seeds are the Azadirachtins (Isomers A-G) (REMBOLD et al., 1987a,b; REMBOLD, 1989, 1990).

Much lesser interest was given to the Chinaberry, *Melia azedarach* L., belonging to the same plant family. The tree is more distributed in subtropical regions. It can be found also in South Europe cultivated in gardens, parks and along streets.

The less application of *Melia* extracts in plant protection based on some information that *M. azedarach* was sometimes toxic to mammals and birds (STEYN and RINDL, 1929; MORRISON and GRANT, 1932; MORTON, 1982). But the toxicity seems to be dependent on the region, from which the fruits were collected (OELRICHS et al., 1983). Other studies could not find toxic effects (HURST, 1942; SCHULTE et al., 1979). BHANDERI and GOVIL (1978) showed that the leaves are of special nutritive value for sheeps and goats. Also AHMED et al. (1984) reported that the tree is a

1) The experiments were carried out at LG Zoologie-Entomologie, FB Biologie, University of Hannover, F. R. G. and were funded by the Scientific Channel System between the Arab Republic of Egypt and the Federal Republic of Germany.

source of animal food. Seeds of *M. azedarach* are eaten by various species of birds.

Basing on the investigations of BREUER and SCHMIDT (1995, 1996) the present work includes studies on the evaluation of the effect of *M. azedarach* extract on two lepidopteran species which are main insect pests in Egypt. Moreover, an enriched extract was used to increase the effectiveness.

2 Material and Methods

2.1 Rearing of the test insects

The initial material (pupae) were obtained from laboratory strains raised in the Plant Protection Department of the National Research Centre, Cairo, Egypt. *Spodoptera littoralis* (Boisd.) and *Agrotis ipsilon* (Hufn.), were reared under constant conditions at the Department of Zoology-Entomology, University of Hannover (Temp. 25 ±3 °C, 40–50% R. H. and normal day light).

2.1.1 Spodoptera littoralis

About 100 pupae were placed in plastic cages (28 × 28 × 17 cm), where the adults emerged 10–15 days later and started egg laying after further 3 days. They were fed on 15% honey solution, which was changed every 2 days. The sides and cover of the cages were lined with sheets of waxy paper to serve as an egg-laying substrate for the females.

Every second day the egg masses were collected. Five masses were placed together in a plastic box (7.5 cm diam) with an artifical diet. These boxes were kept upside down and covered with stuff material. The larvae hatched within 3–4 days and crawled up the sides of the box to feed on the diet.

When the larvae reached the 3rd instar they were transferred to larger plastic cages (45 × 26 × 28 cm), covered with gauze. Each cage contained 150 larvae. The bottom was covered with sheets of towel paper in order to absorb excessed humidity. Four pieces of the prepared artificial diet (approximately 200 g in total) were placed in Petri dishes in each cage, which were replaced from time to time depending on the size and age of the larvae. When the larvae pupated, they were collected and placed in cages for emergence.

2.1.2 Agrotis ipsilon

The pupae were kept in glass jars and covered with gauze until the adults emerged after 15–20 days. Usually, the males appeared 1–3 days earlier than the females. As soon as the adults emerged, they were transferred to bigger jars (10 cm diameter, 10 cm high). Six males and 6 females were placed in each container which was filled with onion-skin paper and covered with black gauze. The adults were fed on 15% honey solution which was renewed every second day. The females started egg laying three days after emergence.

Every second day, the paper and the gauze, on which the eggs had been deposited, were transferred to new jars. The young larvae hatched after 5 days. For the first 5 days, the larvae were fed on hydroponic wheat shoots, cultivated in the laboratory, because mortality was found to be very high among the first instar larvae, when fed on artificial diet. When the larvae reached the second instar, a piece of about 40 g of the diet was placed in the jar. After 9–10 days, the 3rd instar larvae were transferred singly in small vials (3.5 cm diam, 8 cm high) with foam stoppers, because *A. ipsilon* started to exhibit a cannibalistic behaviour. Every week a small piece of about 20 g of the diet was placed in each vial. Pupation started after about three weeks.

2.1.3 Food materials

Honey solution: A 15% honey solution soaked in cotton batches was used to feed the adult moths of both species.

Wheat germ: The first instar larvae of *A. ipsilon* were fed on wheat shoots which were hydrocultured in special pots in the laboratory.

Artificial diet: The diet was prepared as described by KHALIFA et al. (1973) and contained dried kidney beans, agar-agar, yeast, ascorbic acid, sorbic acid, benzoic acid, HCl 10%, formaldehyde 40% and a vitamin mixture.

2.2 Preparing of Melia extract

The fruits were collected from trees of *Melia azedarach* L. in Greece and stored in a deep freeze.

In order to prepare the extract, the following procedure was conducted: 50 g of frozen fruits were ground with 100 ml 80% methanol in a blender. This procedure was repeated twice and the extract was transferred to a glas beaker, stirred for one hour and filtered under low pressure.

The residue was extracted again, using the same procedure. The extracts were combined, filtered and transferred in clean flasks with known weight. The extract was concentrated by using a rotatory evaporator (waterbath at 35 °C) and lyophilized for 24 h. The weight of the residue was calculated. The dry extract was redissolved in the tenfold amount of 80% methanol and washed with the same volume of petroleum ether (b.p. 30–50 °C) by stirring for half an hour.

After separation of the layers using a funnel, the methanol extract was again stirred with half of the volume of the petroleum ether used before. The methanol extract was again separated, evaporated *in vacuo* and lyophilised for 24 h. Then, the dry weight was estimated. Distilled water (10 times of the dry weight of extract) and the same volume of ethyl acetate was added and the mixture was stirred for half an hour.

The solution was left in a separation funnel. After 24 h, the water extract was washed with half of the volume of ethyl acetate used before, and separated again. The ethyl acetate extract was brought to dryness and redissolved in 80% methanol giving a final concentration of 10% (w/v).

2.3 Food consumption experiments

To study the effect of *Melia azedarach* extract on the food consumption of the larvae of both species, 3 concentrations were used (10, 50, 100 ppm). The experiments were started with twenty five 3rd instar larvae for each concentration and an equal number as control (diet mixed only with the solvent). The larvae were weighed before the beginning of the experiment. A group of larvae with the same weight were dried up to determine the dry weight. After 10 days the weight of larvae, rest of food and fecal pellets were recorded for each larva. Nutritional indices were calculated using the following formulae after WALDBAUER (1968):

Relative growth rate:

$$RGR = \frac{(G)}{(T) \times (A)}$$

G = Dry weight gain during feeding period, T = Duration of feeding period (days), A = Mean dry weight of animal during feeding period.

Approximate digestibility:

$$AD = \frac{\text{amount ingestedfood (mg)} - \text{excreta (mg)}}{\text{amountingestedfood (mg)}} \times 100$$

Efficiency of conversion of digested food:

$$ECD = \frac{\text{weight gain (mg)}}{\text{amount ingested food (mg)} - \text{excreta (mg)}} \times 100$$

Efficiency of conversion of ingested food:

$$ECI = \frac{\text{weight gain (mg)}}{\text{amount ingested food (mg)}} \times 100$$

It is worthy to mention that these calculations were based on dry weight and weight of food per larva at the beginning of the experiment.

To calculate the rate of antifeeding activity of *Melia* extract on the target insects, the following formula was used according to SALEH et al. (1986):

$$\text{Antifeeding activity} = (1 - \frac{\text{\% of treated food consumed}}{\text{\% of non treated food consumed}}) \times 100$$

2.4 Determination of larval and pupal weight and insecticidal effects

For this experiment, twenty five 3rd instar larvae (6 days old in *Spodoptera littoralis* and 9 days in *Agrotis ipsilon*) were used for each concentration (10, 15, 25, 50, 100 and 1000 ppm). The same number of larvae were fed on normal diet and used as control. Treated and untreated larvae were kept singly in Petri dishes (12 cm diam). The weight of larvae and pupae were recorded daily.

The same concentrations (10 3rd instar larvae each) mentioned before were used to determine the effect on the percentage of mortality during larval and pupal period. These experiments were carried out in five replicates.

2.5 Determination of various reproduction parameters

Ten pairs of virgin moth that emerged from the larvae previously treated with different concentrations of *Melia* extract (10, 15, 25 ppm) and from control, were used to determine the following parameters: lifespan, pre-oviposition period, oviposition period, post-oviposition period, total number of eggs, number of larvae hatched, and eggs/treated female

$$\text{percentage of fecundity} = \frac{\text{eggs/treated female}}{\text{eggs/untreated female}} \times 100$$

2.6 Histological technics

The 4th instar larvae of both species were examined after 3 days feeding on treated diet (100 ppm *Melia* extract) and compared with same instar larvae which fed on untreated diet as control.

The larvae were fixed in alcoholic Bouin's solution for 24 h, washed in 70% ethanol for one hour (two replacements) and then transferred to 96% ethanol for one hour followed by two replacements in absolute ethanol for 4 h. Then, they were put to a mixture of methylbenzoate/benzol (1:1) for 3 h (3 replacements) and after that to benzol for 15 min followed by a mixture of equal parts of benzol and paraffin for 30 min. The samples were transferred to pure paraffin (p.p. 56–58 °C) (24 hours, 2–3 times renewed) and embedded in paraffin blocks (ROMEIS, 1968). Sections of 5 µm were prepared, stained with Ehrlich's haematoxylin and counterstained with methylene blue or eosine Y 234.

2.7 Statistical analysis

For statistical analysis the F-test, interaction F-test and t-test were used.

3 Results

3.1 Effect of Melia extract on food consumption

3.1.1 Spodoptera littoralis

Table 1 shows no significant difference in food consumption between larvae in control (597.0 mg) and larvae treated with 10 ppm *Melia* extract (518.9 mg). The difference was significant between control and treatment with 50 ppm (431.6 mg), and highly significant with 100 ppm extract (157.4 mg).

The amount of produced feces was accordingly less at the treatment with 50 ppm and 100 ppm extract than in control. The percentage of feces in relation to food consumption was 54.1% in control, 55.7% with 10 ppm, 49.5% with 50 ppm and 22.0% with 100 ppm *Melia* extract.

The weight gain of larvae decreased significantly already when treated with 10 ppm.

The approximate digestibility (AD) increased and the efficiency of conversion of digested food (ECD) decreased significantly in larvae treated with high concentrations of *Melia* extract.

The efficiency of conversion of ingested food (ECI) decreased significantly with increasing *Melia* extract concentration, too. Antifeeding activity of *Melia* extract on 3rd instar larvae of *S. littoralis* was computed as 11.2% at 10 ppm, 18.2% at 50 ppm and 56.9% at 100 ppm.

The relative growth rate (RGR) was affected significantly with 100 ppm (0.178) when compared with control (0.198).

3.1.2 Agrotis ipsilon

The food consumption of treated *A. ipsilon* larvae differed from control at 50 ppm and 100 ppm treatment (table 2). The food consumed was 377.1 mg in control and 346.1 mg at 10 ppm, while it was 270.7 and 222.9 mg at 50 and 100 ppm application of *Melia* extract, respectively.

The production of feces of treated larvae decreased significantly with increasing concentration of *Melia* extract

Table 1. Effect of different concentrations of *Melia* extract on food consumption of *Spodoptera littoralis* larvae; for abbreviations see Material and Methods

Nutritional parameters	Control	Extract concentrations		
		10 ppm	50 ppm	100 ppm
Food consumed (mg)	597.0±35.0	518.9±30.6	431.6±58.6	157.4±7.4
Feces (mg)	322.9±18.3	289.2±21.9	140.8±15.9	34.6±3.6
% of feces to food consumption	54.1%	55.7%	32.6%	22.0%
Weight gain (mg)	149.2±9.4	103.8±3.4	62.4±10.6	14.4±1.6
AD	44.5±3.2	44.1±2.1	62.5±3.5	87.3±1.9
ECD	61.6±5.7	48.6±3.8	25.0±4.2	11.5±1.0
ECI	26.0±2.2	20.7±1.1	14.4±1.9	8.9±0.7
Antifeeding activity		11.2%	18.2%	56.9%
RGR	0.198±0.002	0.197±0.002	0.192±0.002	0.178±0.002

Table 2. Effect of different concentrations of *Melia* extract on food consumption of *Agrotis ipsilon* larvae; for abbreviations see Material and Methods

Nutritional parameters	Control	Various concentrations of *Melia* extract		
		10 ppm	50 ppm	100 ppm
Food consumed (mg)	377.1±17.7	346.1±11.2	270.7±13.2	222.9±6.4
Feces (mg)	179.5±10.4	84.7±10.8	34.7±3.0	10.2±2.6
% of feces to food consumption	47.6%	24.5%	12.8%	4,6%
Weight gain (mg)	42.4±2.7	23.0±2.4	14.4±2.1	4.6±0.7
AD	51.6±2.9	76.4±2.4	87.4±0.7	95.6±1.0
ECD	24.3±2.9	8.8±0.9	6.2±0.7	2.2±0.3
ECI	11.6±1.1	6.5±0.5	5.4±0.6	2.1±0.3
Antifeeding activity		7.7%	32.1%	51.1%
RGR	0.19±0.001	0.18±0.002	0.17±0.003	0.14±0.008

Table 3. Effect of different concentrations of *Melia* extract on total mortality (in larval and pupal stage) and pupal period

Treatments	Control	Extract concentrations					
		10 ppm	15 ppm	25 ppm	50 ppm	100 ppm	1000 ppm
Spodoptera littoralis							
Mortality (%)	8	16	20	36	100	100	100
pupal period (days)	10.5±0.3	10.0±0.4	10.8±0.3	10.0±0.4	–	–	–
Agrotis ipsilon							
Mortality (%)	8	28	40	64	88	100	100
pupal period (days)	14.6±0.2	17.3±0.3	21.5±0.5	19.0±0.7	20.3±0.7	–	–

in the diet, from 179.5 mg in control to 10.2 mg in larvae treated with 100 ppm extract.

The percentage of feces, according to food consumption, decreased if *Melia* extract concentration was increased. It was 47.6% in control, 24,5% at 10 ppm, 12.8% at 50 ppm and 4.6% at 100 ppm.

The weight gain of treated larvae was very low when treated with 100 ppm (4.6 mg) and highly significant if compared with the control (42.4 mg) and the lower *Melia* extract concentrations .

A significant increase is shown also in the approximate digestibility (AD) up to 100 ppm *Melia* extract. The efficiency of conversion of digested food (ECD) was also affected significantly at the different concentrations (10, 50 and 100 ppm) compared with the control. Between the various concentrations there was no significant difference up to 50 ppm. Differences were also found in the efficiency of ingested food (ECI) between control and different *Melia* extract concentrations.

The antifeeding activity of Melia extract was 51.1% in 100 ppm, and 23.1% in 50 ppm. It was very low in 10 ppm (7.7%).

The relative growth rate (RGR) was found to be significantly changed by *Melia* extract at 100 ppm.

3.2 Insecticidal effect of Melia extract

3.2.1 Spodoptera littoralis

Data presented in table 3 show that the toxic effect of enriched methanolic extract of *M. azedarach* was very

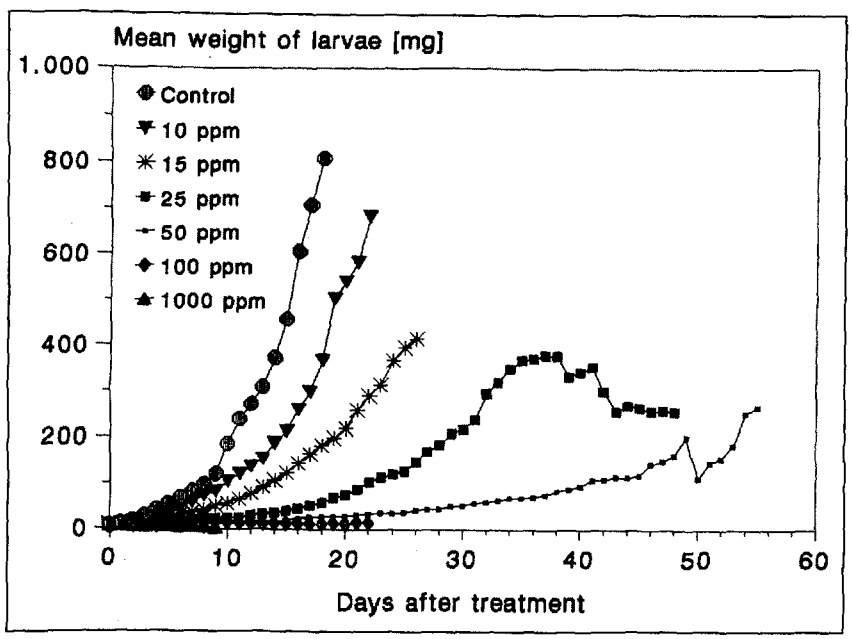

Fig. 1. Effect of different concentrations of *Melia* extract on the weight of *Agrotis ipsilon* larvae until prepupal stage or mortality

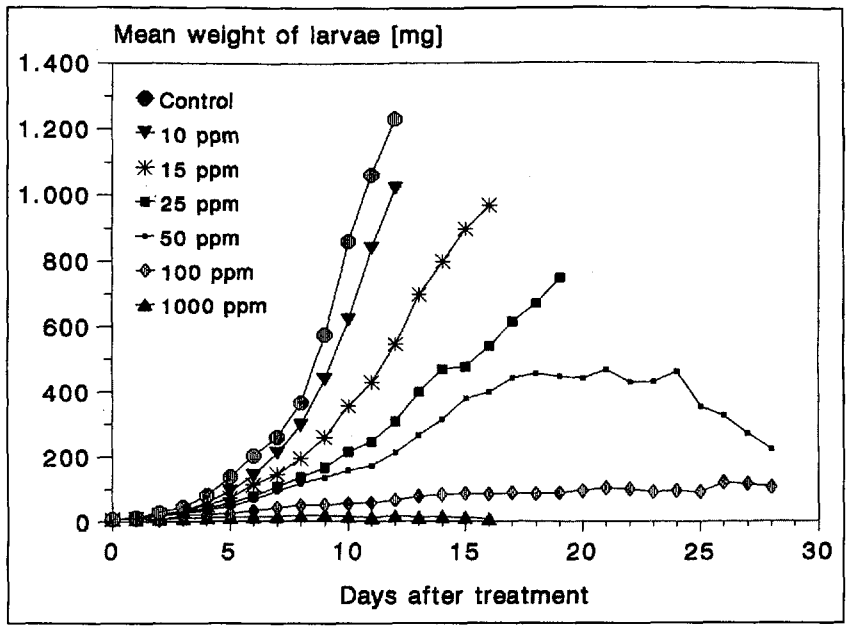

Fig. 2. Effect of different concentrations of *Melia* extract on the weight of *Spodoptera littoralis* larvae until prepupal stage or mortality

high at concentrations of 50, 100 and 1000 ppm. All insects died in these experimental series during larval and pupal stage, while at 10, 15 and 25 ppm the mortality was only 16, 20 and 36%, respectively. In the control 8% mortality was observed. Pupal duration was not affected.

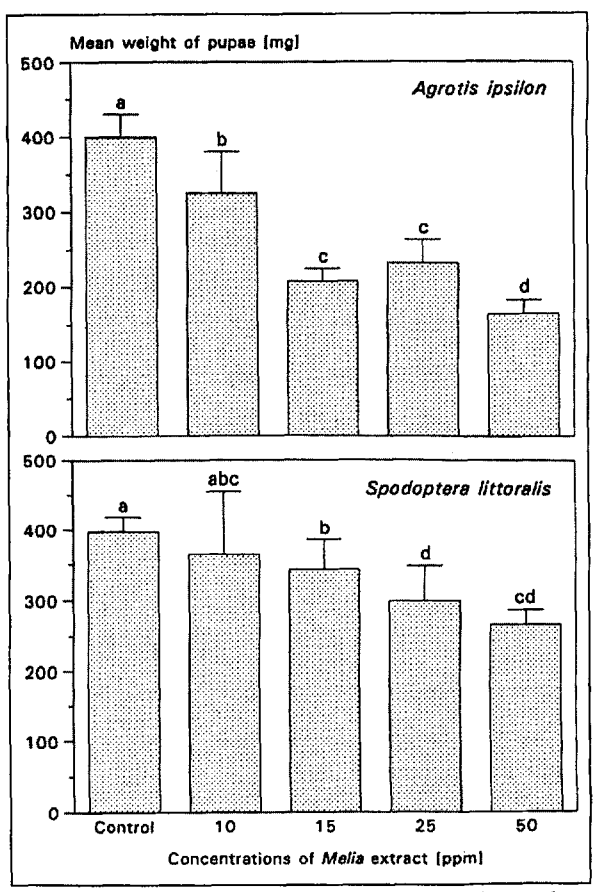

Fig. 3. Effect of *Melia* extract on the pupal weight; letters show the differences on $P < 0{,}01$ level

3.2.2 Agrotis ipsilon

Melia extract was also toxic to the 3rd instar larvae of *A. ipsilon;* at 25 ppm 64%, at 50 ppm 88% and at 100 and 1000 ppm 100% mortality was observed (table 3). In the control, and at lower extract concentrations less mortality was found. Pupal duration was prolonged with increasing *Melia* extract concentrations. During the experiments with both insect species, it could be observed that *Melia* extract induced different malformations and moulting defects.

3.3 Effect of Melia extract on larval and pupal weight

3.3.1 Weight of larvae

Fig. 1 shows that the weight of treated larvae of *A. ipsilon* was affected by the extract of *Melia* depending on its concentration. Sixteen days after experimental start significant differences could be found between control and treated larvae. The mean larval weight was 602 mg in control, 260 mg at 10 ppm, 143 mg at 15 ppm, and below 50 mg in higher extract concentrations.

In the case of *S. littoralis* (fig. 2), significant differences were observed between the weight of larvae in control and that at different *Melia* extract concentrations, 12 days after starting of the experiment. The mean weight of the control larvae was 1228 mg and 16 mg, if 1000 ppm extract concentration was used. Only between control insects and those treated with 10 ppm extract, no significant difference was found in the mean weights of larvae.

3.3.2 Weight of pupae

In *A. ipsilon* and *S. littoralis*, pupal weight was affected by the different concentrations of *Melia* extract (fig. 3). In the black cutworm the mean weight of pupae was 400 mg in control, 327, 209, 234 and 165 mg in insects treated with 10, 15, 25 and 50 ppm extract, respectively. The mean weight of pupae of the cotton leafworm was also reduced from 397 mg in control to 343 mg, 300 mg and 267 mg at 15, 25 and 50 ppm extract, respectively. At higher concentrations no larva reached the pupal stage.

3.4 The effect on larval and pupal periods

3.4.1 Spodoptera littoralis

The mean periods from 3rd larval instar to pupation ranged between 15.3 days in the control and 23.8 days at 50 ppm extract (fig. 4). No significant difference was found between 25 and 50 ppm *Melia* extract. Pupal period was not affected at different concentrations when compared with the control (table 3).

3.4.2 Agrotis ipsilon

Data presented in fig. 4 show that the period between the start of the experiment and the pupation was significantly prolonged by higher extract concentrations than 10 ppm. The mean periods until pupation were 24.0 in control, 25.4, 34.9, 47.6 and 60.3 days for concentrations of 10, 15, 25 and 50 ppm, respectively.

Pupal period was also affected using diet with *Melia* extract during larval development (table 3). The pupal stage lasted on the average 14.6 days in control and increased to 20.3 days at 50 ppm extract concentration. Between the concentrations of 15, 25 and 50 ppm there was no significant difference in pupal periods, while the increase was significant when compared with the pupal durations at 10 ppm and the control.

3.4.3 Effect of Melia extract on various reproduction parameters

3.4.3.1 Effect on adult longevity and oviposition period

Spodoptera littoralis

Male longevity was reduced with increasing *Melia* extract concentration; in case of 10 ppm the difference was not significant to the control (table 4). The same effect was found in females. The mean lifespan lasted 14.8 days in control and only 6.6 days at 25 ppm extract. The decrease concerned mainly the oviposition and post-oviposition period, while the pre-oviposition period was little longer in females treated during larval development.

Agrotis ipsilon

Male and female longevity were similar sensitive (table 4). At higher concentrations, both sexes were affected significantly when compared with the control. The mean durations of lifespan were 14.8 days for males and 19.4 days for females at control, and 5.2 and 7.0 days at 25 ppm, respectively.

In *A. ipsilon* females the oviposition period was significantly reduced, too. A little decrease could be seen also in the post-oviposition period. The pre-oviposition period was not affected.

3.4.3.2 Effect on fecundity, fertility and percentage of sterility

Spodoptera littoralis

Table 4 shows a reduction in the mean number of eggs/female (fecundity) with increasing *Melia* extract concentration from 4116 eggs in control to 1009, 163 and only 9 eggs at 10, 15 and 25 ppm extract, respectively. This is only 24.5%, 4.0% and 0.2% of the fecundity of the control. The fertility (number of hatched larvae/female) was reduced significantly at 10 and 15 ppm. At 25 ppm no larvae occurred. The percentage of hatching

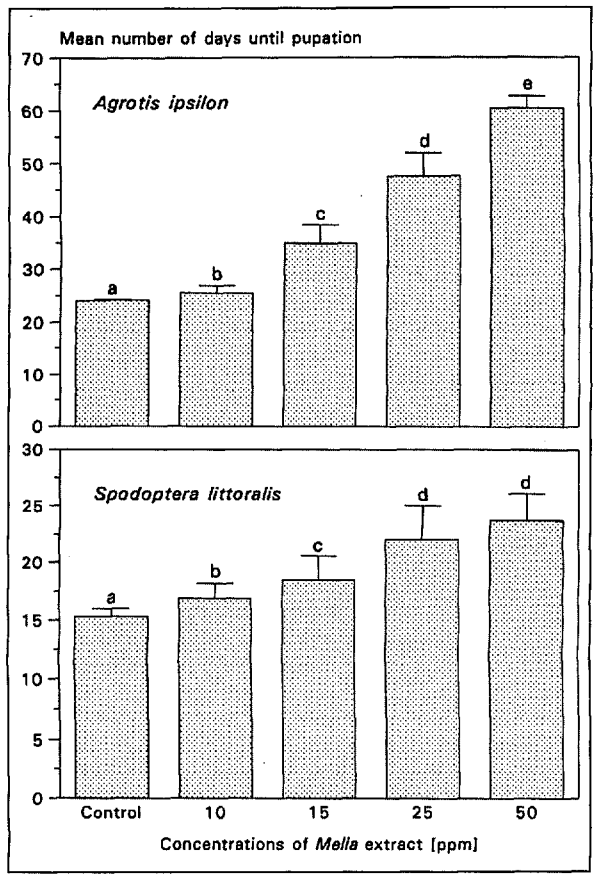

Fig. 4. Effect of *Melia* extract on the period from 3rd instar larva until pupation; letters show the differences on $P < 0{,}01$ level

decreased from the control to 10 and 15 ppm extract concentration. The sterility effect of the extract was 98.7% at 15 ppm and 77.5% at 10 ppm in relation to the control.

Agrotis ipsilon

The mean number of eggs per female was reduced significantly at the tested concentrations when compared with the control as demonstrated in table 4. At 25 ppm, on the average, only 12 eggs/female were layed, while this number in control was 1274 eggs. The fecundity of the females in treatments calculated as percentage of the control was 59.7% at 10 ppm, 10.7% at 15 ppm and only 0.9% at 25 ppm. The hatchability was reduced from 1133 larvae in control to 569 larvae at 10 ppm. The fertility decreased significantly with higher concentrations from 113 larvae at 15 ppm to only 2 larvae at 25 ppm, that means that only 53 and 17% of the eggs produced larvae.

The sterility effect of the extract on the adults which emerged from treated larvae was 49.2% at 10 ppm and increased to 93.5% at 15 ppm and 99.8% at 25 ppm *Melia* extract.

3.5. Effect on midgut epithelium

The toxic ingredients of the *Melia* extract have to pass the midgut before affecting other processes. Therefore, cross sections of the midgut of the 4th larval instar were performed after feeding a diet mixed with 100 ppm *Melia* extract for three days in comparison with larvae of the

Table 4. Effect of *Melia* extract on some biological parameters of *Spodopera littoralis* and *Agrotis ipsilon.* Significances to the control: * $P < 0.05$, ** $P < 0.01$

Biological parameters	Control	Extract concentrations		
		10 ppm	15 ppm	25 ppm
Spodoptera littoralis				
Male longevity	14.4±4.3	12.0±5.0	7.2±3.1*	3.6±1.7**
Female longevity	14.8±0.8	11.6±5.2	10.0±2.6*	6.6±3.3**
Pre-oviposition period (days)	2.4±0.9	4.4±0.9*	4.2±1.6*	4.6±2.7*
Oviposition period (days)	9.2±2.3	5.6±4.7	3.8±2.2**	1.6±2.1**
Post-oviposition period (days)	3.2±1.8	1.6±1.1	2.0±1.0	0.4±0.6*
Number of eggs laid/female	4116.2±2804.7	1009.2±913.4*	163.2±87.0*	9.4±12.2**
Number of hatched larvae	3369.6±2204.9	839.6±819.4*	51.2±49.2**	–
Percentage of hatching	85.7±9.6	78.7±10.0	28.6±15.0**	–
% Fecundity, compared to control		24.5	4.0	0.2
% Sterility, compared to control		77.5	98.7	
Agrotis ipsilon				
Male longevity	14.8±1.7	9.2±0.8*	6.4±1.6**	5.2±1.5**
Female longevity	19.4±1.7	14.0±1.6	6.2±0.7**	7.0±1.9**
Pre-oviposition period (days)	3.4±.7	2.6±0.7	3.0±0.9	2.8±0.7
Oviposition period (days)	11.4±1.0	6.4±1.1**	2.0±0.9**	2.2±0.8**
Post-oviposition period (days)	4.6±1.1	5.0±1.5	1.2±0.6*	2.0±0.7*
Number of eggs laid/female	1274.0±320.2	760.8±143.1*	136.4±61.3**	12.0±7.2**
Number of hatched larvae	1133.4±292.0	569.8±104.6*	113.7±6.7**	2.0±0**
Percentage of hatching	87.1	74.1	52.9	16.7
% Fecundity, compared to control		59.7	10.7	0.9
% Sterility, compared to control		49.2	93.5	99.8

same stage fed with a diet mixed with the same amount of 80% methanol as control.

A great difference was found between sections of treated and untreated larvae of *S. littoralis* (fig. 5). Influenced by *Melia* extract the layers of both longitudinal and circular muscles were separated from the epithelial layer. The peritrophic membrane disappeared and its remains were disposed on the epithelial cells, from which many were destroyed and others seemed to be in a stage of degeneration. The size of these cells became smaller and a space was found between the epithelium and the bulk of food content. Some muscles of the destroyed cells were scattered.

Cross sections of the midgut of *A. ipsilon* showed the same effects after treatment. No difference was observed between the various layers of the midgut.

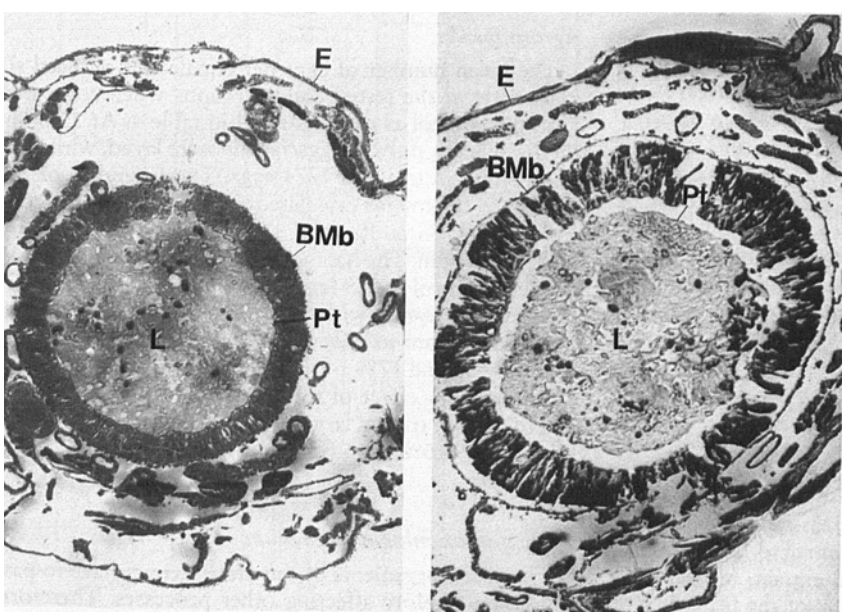

Fig. 5. Cross sections through the midguts of 4th instar larvae of *Spodoptera littoralis;* left: control, right: after three days treatment with 100 ppm of *Melia* extract; L: lumen with food material, BMb: basement membrane, Pt: peritrophic membrane, E: epidermis with cuticle

4 Discussion

The present studies were performed to increase our knowledge on the effect of methanolic extracts of *Melia* fruits on the growth, development and reproduction of two major pests in Egypt, the Egyptian cotton leafworm, *Spodoptera littoralis,* and the black cutworm, *Agrotis ipsilon.* Nutritional indices calculated according to WALDBAUER (1968) showed that the larvae treated with 10, 50 and 100 ppm *Melia* extract consumed less amounts of food with increasing extract concentrations. The dry weight of feces, weight gain and growth rate were positively affected. The cross sections of the gut of the 4th instar of both target species treated by 100 ppm *Melia* extract approved that the epithelial cells of midgut were heavily destroyed, thus digestion and absorption of food may be restrained. The separation of the muscle layers from the epithelium indicated that the treated insects lost the ability to transport the uptaken food through the gut and consequently feeding was reduced which agrees with the findings

of KRAUS et al. (1981) that *Melia* extract has an antifeedant effect. BREUER and SCHMIDT (1995) showed that larvae of *Spodoptera frugiperda* have to take up the extract substances only along with food, because there was no significant effect caused by merely contact.

The approximate digestibility (AD), measuring the assimilation of food was increased significantly at extract concentrations higher than 50 ppm in both insect species. ARNASON et al. (1987) mentioned that AD somewhat increased after treatment with some limonoids from Meliaceae when tested against *Ostrinia nubilalis* larvae. The efficiency of conversion of digested food (ECD), indicating the percentage of assimilated food converted into body matter, was reduced at all extract concentrations tested. The same effect was observed for the efficiency of conversion of ingested food (ECI) measuring the overall ability of the insect to convert ingested food into body matter. Also these calculations demonstrated that *Melia* extract has strong effects on the absorption of ingested food and consequently on the conversion into larval tissues.

RAO and SUBRAHMANYAM (1986) studied the effect of azadirachtin on food utilization of *Schistocerca gregaria* and found that this compound reduced feeding at about 50% in both males and females.

The reduced growth rate depending on lesser food utilization was mainly observed in treated females. Insignificant growth rate in males suggests that food consumed was largely utilized for maintenance and only very little for growth.

The 3rd larval instar of *S. littoralis* was little more sensitive to the *Melia* extract than those of *A. ipsilon*. At 50 ppm *Melia* extract pupation was much lower (16%) in *S. littoralis* than in *A. ipsilon* (75%); from the former no adults emerged. The weight of larvae was significantly reduced after 10 days in *S. littoralis* and 18 days in *A. ipsilon* in experiments with 25 ppm extract. In both species, control larvae grew steadily but treated larvae showed only a slight increase in weight. Thus, retardation in growth in treated larvae of the two species may be due to the role of *Melia* extract in reducing the food consumption as obtained from the data, and this agrees with the present histological results.

BREUER and DEVKOTA (1990) stated that *Thaumetopoea pityocampa* larvae were more sensitive to *Melia* extract than those of *S. frugiperda* (BREUER and SCHMIDT, 1990), when the same concentrations were tested. Having a broad spectrum of detoxification mechanisms polyphagous insects, such as *S. frugiperda* and *A. ipsilon*, seem to be able to metabolize toxic substances easier than oligophagous ones.

The toxic effects depend on the degree of purification of the *Melia* extract. The used methanolic extract, purified through solvent partitioning, was more efficient than that applied in former studies (BREUER and DEVKOTA, 1990; BREUER and SCHMIDT, 1990, 1995, 1996). Already with less active extract quantities up to 100% mortality rates were caused. In spraying experiments, PANDAY et al. (1981) found that the painted bug, *Bagrada cruciferarum*, was very sensitve against dry film emulsions of *Melia* extract. After 24 and 72 hours mortalities of 46.7 and 70% were provided, respectively, at a concentration of 0.5%, and at 2% concentration the mortality was 100%. An aphicidal property of *Melia* extract against 4th nymphal instar of *Aphis fabae* was reported by DIMETRY and SCHMIDT (1991); 100% mortality occurred after 96 hours. Reducing the concentration, the mortality decreased as found in our experiments.

Zusammenfassung

Zur Wirkung von Melia azedarach-Extrakt auf die Larvenentwicklung und Reproduktions-Parameter von Spodoptera littoralis (Boisd.) und Agrotis ipsilon (Hufn.) (Lep., Noctuidae)

Ein methanolischer Extrakt von *Melia azedarach* – Früchten aus Griechenland wurde auf seine insektizide Wirkung bei Raupen zweier schädlicher Lepidopteren (*S. littoralis* und *A. ipsilon*) unter Laborbedingungen untersucht. Der Extrakt wurde zuvor mit Petrolether und Ethylacetat gereinigt. Für die Experimente wurden neben der Kontrolle Konzentrationen von 10, 15, 25, 50, 100 und 1000 ppm mit einer Diät verfüttert. Bei beiden Insektenarten sank die Nahrungsaufnahme, die Gewichtszunahme und die Umwandlung der aufgenommenen Nahrung (ECI) in Biomasse mit zunehmender Extraktkonzentration in der Nahrung ab. Der Verbrauch der verdauten Nahrungsmenge (ECD) wurde mit höherer *Melia*-Extraktmenge graduell geringer. Sowohl bei den Raupen von *S. littoralis* als auch bei *A. ipsilon* wurde ein gewisses Antifeedant-Verhalten beobachtet. Die Mortalität stieg mit erhöhter *Melia*-Konzentration in der Diät bei beiden Arten an. Mit Beginn des 3. Larvenstadiums wurde das Gewicht bei beiden Arten bis zur Verpuppung bei 25 ppm und höheren Konzentrationen signifikant reduziert, während die Larvalperiode verlängert wurde. Das Puppengewicht war signifikant niedriger, wenn Diäten mit Konzentrationen von 15, 25 und 50 ppm *Melia*-Extrakt verabreicht wurden. Bei höheren Konzentrationen verpuppten sich die Larven nicht mehr. Die Puppenperiode wurde nur bei *A. ipsilon* beeinflußt. Alle die Reproduktion betreffenden Parameter, wie Eiablagezeit, Fekundität, Fertilität und Lebenszeit der adulten Männchen und Weibchen, wurden beeinflußt, wenn die Larven mit Konzentrationen von 10, 15 und 25 ppm *Melia*-Extrakt aufwuchsen. Bei *S. littoralis* schlüpfte kein Falter, wenn die Raupen mit 50 ppm und mehr gefüttert wurden, und keine Larve schlüpfte aus Eiern, die von Weibchen abgelegt wurden, die als Larven 25 ppm *Melia*-Extrakt in der Diät erhielten. Bei beiden Arten war die Ovipositionszeit verkürzt, wenn ihnen 15 und 25 ppm *Melia*-Extrakt mit der Diät verabreicht wurden; die Fekundität und Fertilität waren stark reduziert, und die Lebenszeit war sowohl bei Männchen als auch bei Weibchen verkürzt. Querschnitte durch den Mitteldarm zeigten, daß bei Raupen beider Arten, die mit 100 ppm *Melia*-Extrakt in der Diät gefüttert wurden, das Darmepithel stark zerstört war, was als eine Ursache für die beobachteten Effekte angesehen wurde.

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