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INSECT ANTIFEEDANTS AND GROWTH INHIBITORS FROM AZADIRACHTA INDICA AND PLUMBAGO ZEYLANICA

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Solvent extracts of different parts of the neem tree (*Azadirachta indica* A. Juss) were bioassayed for their antifeedant properties against first- and third-instar larvae of *Mythimna separata*. Fraction 'G', obtained from an ethanolic extract of the shade-dried neem seeds (Hyderabad, India) was the most effective phagodeterrent under glasshouse, laboratory and, to a limited extent, field conditions. Fraction 'G' was further purified to fraction 'M', which was subjected to column chromatography (silica gel) resulting in 14 fractions, AI-1 to AI-14. Fractions AI-9, AI-10 and AI-11 were found to be biologically active, reducing leaf feeding and larval weight considerably. None of the treated larvae which fed on leaf discs treated with the fractions and extracts 'G' and 'M' could pupate. The retention time of the major component of AI-10 on a µ BONDAPAK C_{18}, 10 µ, 3.9 mm ID, 30 cm column (high performance liquid chromatography) was close to that of azadirachtin. Spectral investigations revealed that this major component of AI is different from the antifeedants reported earlier from *A. indica* and *Melia azedarach*. It has been designated as vepaol (the Telugu name for neem is vepa), and some aspects of its structure are discussed. Studies relating to the effectiveness of fraction 'G' on the oriental armyworm, *Mythimna separata*, and other sorghum pests are presented.

INTRODUCTION

The neem tree (*Azadirachta indica* A. Juss), which grows in the semi-arid regions of the world, is an outstanding example of a species which is not only an efficient soil ameliorant but also a source of several useful products, such as timber oil, fertilizer, nitrification inhibitor, and pesticides. The mixing of neem leaves with stored grains is an age-old practice in India. Pruthi (1937) reported the first scientific data on the repellent action of neem leaves against storage pests. The demonstration of the antifeedant properties of the neem kernel against the desert locust, *Schistocerca gregaria* (Pradhan *et al.*, 1962), generated tremendous interest in the insect controlling properties of this plant.

The diverse biological effects of the neem kernel extracts are phagodeterrent and repellence (Pradhan *et al.*, 1962; Joshi & Ramaprasad, 1975; Jacobson *et al.*, 1978; Ladd *et al.*, 1978; Warthen *et al.*, 1978a, b), growth inhibition and abnormal development (Leuschner, 1972; Meisner *et al.*, 1976, 1978; Redfern *et al.*, 1979;

Schmutterer & Rembold, 1980, Sharma *et al.*, 1980; Ascher & Gsell, 1981), and oviposition suppression (Jacobson *et al.*, 1978; Joshi & Sitaramaiah, 1979), which have been amply documented in the Proceedings of the First Neem Conference (Schmutterer *et al.*, 1981).

The biologically active components in neem and the closely related chinaberry tree, *Melia azedarach* L., have been identified as meliantriol (Lavie *et al.*, 1967), azadirachtin (Butterworth & Morgan, 1968; Nakanishi, 1975) and salannin (Warthen *et al.*, 1978b).

Several biologically active constituents have since been isolated from different parts of the neem tree and the closely related chinaberry tree, of which azadirachtin (Fig. 3) is the most potent antifeedant and growth regulator (Kraus *et al.*, 1981; Morgan, 1981; Schmutterer, 1981).

A program was initiated in March 1982 to explore the potential of developing concentrated neem extracts for pest control, using the polyphagous rice ear-cutting caterpillar, *Mythimna separata*, as a test insect. The insect had earlier been found to respond to neem extracts (Sharma *et al.*, 1982). This paper reports isolation of a new triterpenoid, vepaol - closely related to azadirachtin - from the biologically highly active fraction, and the activity of the crude extracts and some fractions under both laboratory and field conditions.

MATERIALS AND METHODS

Extraction and fractionation

In separate experiments, neem seeds collected in July 1981 and kernels collected in June - July 1982 at Hyderabad, India, were crushed in hexane petroleum ether (bp 60 - 80 °C) with a Sumeet domestic mixer and the extracts were filtered and concentrated in vacuum. Simple solvent fraction of the concentrate from the ethanolic extract yielded the crude biologically highly-active solid fraction designated as 'G'. Fraction 'G' was further purified to fraction 'M'. Fraction 'M' was subjected to column chromatography over silica gel, resulting in 14 fractions designated as AI-1 to AI-14. Fraction AI-10 was purified by repetitive column chromatography (silica gel), and semi-preparative (μ Bondapak C18/Porasil B, 37,35 μ, 2 mm ID x 61 cm) and analytical (μ Bondapak C18, 10 μ, 3.9 mm ID x 30 cm) high performance liquid chromatography (Detector, UV, 217 nm; eluent MeOH : H_2O, 50 : 50). Extracts of unripe seeds and the flowers collected in April 1981 at Hyderabad were prepared by refluxing thrice with 95% ethanol, filtering, and concentrating the combined extracts in vacuum.

Plumbagin, a pure compound obtained from *Plumbago zeylanica*, was tested along

with the neem extracts. In another experiment (Table III), hexane and alcoholic extracts of leaves, acetone extract of unripe seeds, and fraction 'E' obtained from the ethanolic extract of kernels and whole seeds were tested for their biological activity.

An ethanolic extract of the neem kernel was also partitioned through methanol-water (9 : 1) and petroleum ether (bp 80 °C) to obtain biologically enriched extracts.

Bioassay techniques

Various extracts and fractions were bioassayed under laboratory and glasshouse conditions. Fraction 'G' was also tested under field conditions. The various techniques adopted for bioassay are described below:

Bioassay on potted plants: Plants of pearl millet hybrid BJ-104 were grown in 15-cm-diam plastic pots in a mixture of Alfisol and farmyard manure (1 : 1) in the glasshouse. The 10-day-old plants were thinned to ten plants of uniform growth in each pot. A 10-ml solution of the extracts in the appropriate solvents (methanol : water (8 : 2), acetone or hexane) was sprayed with the help of a graduated hand atomizer onto five pots kept in a row. One hour after spraying, 1st-instar or 3rd-instar larvae of *M. separata* were released into each pot. The larvae were confined to the treated pots with the help of a plastic cage (11 cm diam, 25 cm long) having four wire mesh ventilation openings (5 cm diam) on the sides and one at the top. The larvae were removed from the treated pots after 3 - 5 days, when the damage (leaf feeding) in the control pots exceeded 80%. The pots were rated visually for the extent of leaf feeding on a scale of 1 to 5 (1 = 10%, 2 = 11 to 25%, 3 = 26 to 40%, 4 = 41 to 60%, and 5 = > 60% leaf area consumed). The larvae were placed individually in glass vials and weighed after 2 h. In some cases the larvae were reared on their natural food (pearl millet leaves) until pupation. Observations were made of the duration of larval and pupal development. The moths emerging from each treatment were put in a 30 x 30 x 30 cm cage, and provided with 10% honey solution. Greaseproof paper sheets were put on the sides of the cage for egg-laying; eggs laid were counted daily, and the number of larvae hatched were also counted. The unconsumed leaves from the treated and control pots were collected in polyethylene bags and their surface measured with a leaf area meter. The percentage leaf area consumed was calculated in relation to the check pots without larvae. The leaves treated were then dried in an oven at 80 °C for 5 days, after which the dry weight was recorded.

Leaf disc assay: Two sizes (13.82 and 5.85 cm^2) of leaf discs of uniform diameter, cut out with the help of sharp rimmed iron pipes from the same position on the sorghum or pearl millet leaves, were used in these tests. Each leaf disc was dipped (for 5 sec) in 2 ml of the respective extract solutions and allowed to dry on filter paper for 1 h. After drying, the larger leaf discs were offered in 9-cm-diam plastic petri dishes to two 3rd-instar *M. separata* larvae/leaf disc which had been starved for 2 h in plastic petri dishes. The leaf discs were kept on filter paper moistened with water in petri dishes.

The 3rd-instar larvae were confined to the leaf discs for 48 h, during which period the larvae consumed 80% of the leaf disc area in the untreated control. The smaller leaf discs were provided to 1st-instar larvae in plastic containers. The larvae were removed after 3 days, when > 80% of the green matter in the control discs was scratched. The leaf discs were rated for the extent of feeding on scale of 1 to 5. In some experiments the leaf discs were passed through the leaf area meter, and also dried in an oven at 80 °C for 5 days to record the unconsumed leaf area and the dry weight, respectively. The larvae were placed individually in glass vials and weighed 2 h after starvation. After the exposure period, larvae from different treatments were reared on untreated food to record the developmental period, pupation, emergence and oviposition behavior of the moths emerging from the larvae that had fed on treated leaf discs.

Field assay: Fraction 'G' was tested against the armyworm on pearl millet hybrid BJ-104 in the field during the rainy season, in large unreplicated plots separated by guard rows. Fraction 'G' was formulated as a 20% stock solution in acetone containing 10% emulsifier (Teepol). The solution was mixed with water to give a final concentration of 0.1%, and applied as a spray. Fenvalerate at 0.01% and malathion at 0.1% were also sprayed, for comparison. The sprays were applied twice at 10-day intervals in large plots (90 m^2), using a knapsack sprayer. Third-instar larvae of *M. separata* were released in each plot (750 larvae/plot) before the first spray. The incidence and extent of leaf damage were recorded 25 days after the first spray, through stratified (samples collected from 1 m row at regular intervals of 2 m) sampling. One hundred leaves from each plot were picked at random and evaluated for the incidence and extent of leaf damage. The total leaf damage was computed as a function of the incidence and the damage rating (on a scale of 1 to 5) is presented graphically in Figure 12.

During the rainy season, fraction 'G' was sprayed at 0.1% conch on sorghum. Each neem-treated and control plot (324 m^2/plot each) was surrounded by a buffer zone of 4 m. Each plot was further divided into four sub-plots for observations. The neem extract was applied at weekly intervals, from 20 days after

emergence until physiological maturity of the crop. The data on insect numbers and damage were recorded for shootfly *(Atherigona soccata)*, corn planthopper *(Peregrinus maidis)*, maize aphid *(Rhopalosiphum maidis)*, armyworm *(Mythimna separata)*, headbug *(Calocoris angustatus)*, sorghum midge *(Contarinia sorghicola)*, and the thrips (*Thrips* sp.) at weekly intervals. The observations on each insect were confined to its peak activity period, and were recorded from the center of each plot of ~~from~~ plants selected at random. Eight thousand 3rd-instar larvae of *M. separata* were released in the treated and untreated plots on the 40-day-old crop before spraying. The larval activity and damage were recorded until head emergence.

The *M. separata* larvae were collected one week after their release in the field and reared under laboratory conditions to obtain data on pupation and adult emergence. Observations were also recorded on the parasitization of the armyworm (from the field-collected larvae) and the sorghum midge.

The data were analyzed statistically using analysis of variance to determine the significance of differences between treatments.

RESULTS

Constitution of vepaol

Fraction AI-10 contained one major component, the retention time of which is close so that of azadirachtin. Repetitive column chromatography and semipreparative HPLC resulted in a substance designated as 'vepaol', with a retention time close to that of azadirachtin (Fig. 1). However, it still contained two minor components and further purification is in progress. Vepaol is optically active. Its IR spectrum showed the presence of hydroxyl and carbonyl absorptions ($CHCl_3$ 3575 cm^{-1}, 3400 cm^{-1}, 1738 cm^{-1} and 1695 cm^{-1}). Its mass spectrum showed the presence of significant ions at m/e 659, 658, 559, 521, 83 and 55. Its ^1H NMR spectrum (Fig. 2) showed the presence of a tiglate ester moiety, an acetoxyl, two carbomethoxyl and two quartenary methyl groups, as observed with azadirachtin. A direct comparison of the ^1H NMR spectrum of vepaol with that of azadirachtin (Fig. 3) (Nakanishi, 1975) and revealed subtle differences between the two. A remarkable feature of the NMR spectrum of vepaol is the absence of the signals assignable to the protons of the dihydrofuran ring of azadirachtin (22-H 5.05, d, J = 2.5 Hz; 23-H 6.42, d, J = 2.5 Hz) (Fig. 3) and the absence of signals at 3.43 (S, 3H) and 5.18 (m, 1H) (Fig. 2). Its structure is under investigation.

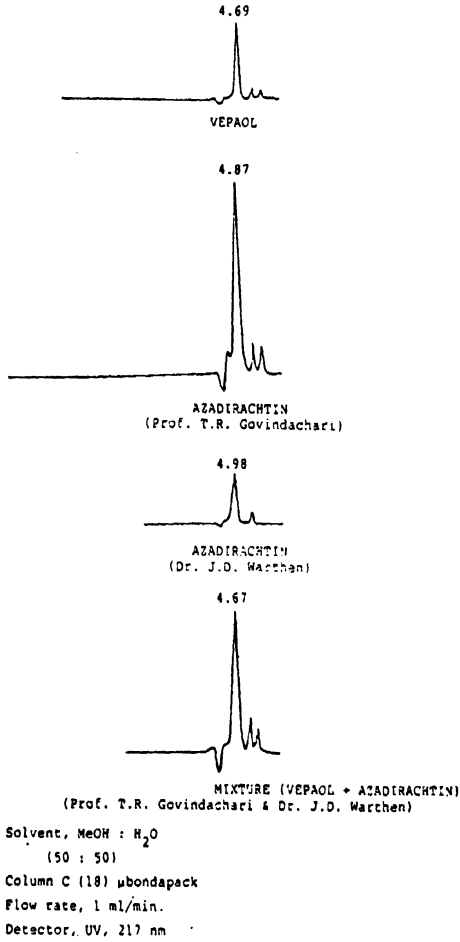

Fig. 1. High performance liquid chromatograph of vepaol and azadirachtin (Retention time in minutes.)

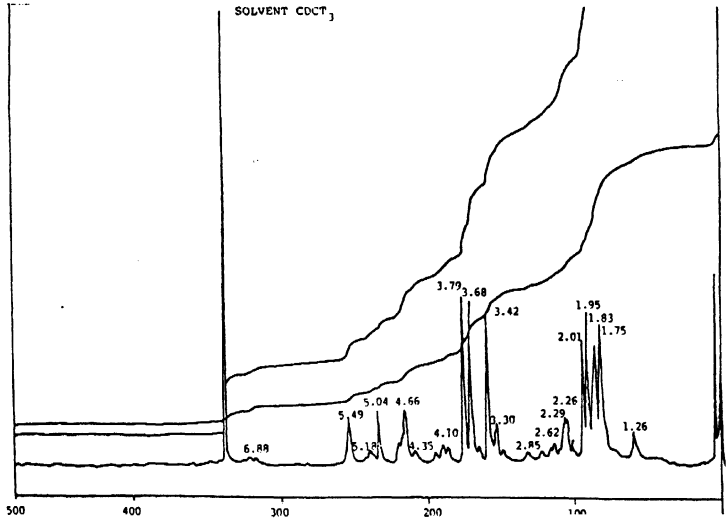

Fig. 2. NMR spectrum of vepaol (89.5 MHz)

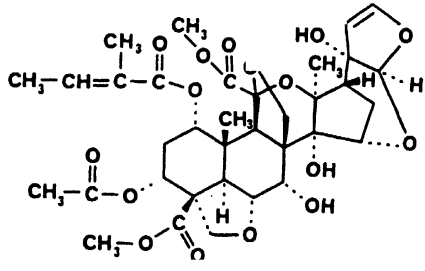

Fig. 3. Azadirachtin

Biological activity of the crude extracts

Effect on feeding: The results of different neem extracts on the 1st- and 3rd-instar larvae of *Mythimna separata* are given in Tables I and II. Fraction 'G' was the most active phagodeterrent in the pot and the leaf disc assay against 1st- and 3rd-instar larvae.

TABLE I

Effect of five neem extracts and plumbagin (all at 0.1% concentration) on the feeding and larval weight of third-instar larvae of Mythimna separata. (Pot assay - Ten larvae were confined with treated seedlings for 3 days (three replications per treatment). Leaf disc assay - Two larvae were confined with the treated leaf discs for 2 days (five replications per treatment).)

Treatment	Pot assay				Leaf disc assay		
	Damage rating*	Dry weight of uneaten leaves (mg)	Larval weight (mg)	Reduction in dry matter consumption (% of control)	Damage rating*	Leaf area consumed (cm^2)	Induction in feeding (% of control)
Shade-dried neem seeds (solid fraction 'G')	1.0	629 (25.1)**	36 (5.9)	66.5	1.6 (1.2)	2.9 (1.6)	61.4
Shade-dried neem seeds (petroleum ether ext.)	3.3	443 (20.9)	81 (9.0)	32.2	3.0 (1.7)	6.3 (2.5)	17.4
Unripe neem fruits (alcoholic extract)	3.5	492 (22.2)	43 (6.5)	41.3	2.4 (1.5)	4.0 (2.0)	46.9
Plumbagin	3.5	404 (20.0)	77 (8.8)	25.0	3.3 (1.8)	6.0 (2.4)	21.4
Shade-dried neem fruits (alcoholic extract)	3.8	353 (18.3)	59 (7.6)	15.7	2.1 (1.4)	3.8 (1.9)	50.8
Neem flowers ((alcoholic extract)	4.0	359 (18.8)	78 (8.8)	16.8	2.4 (1.5)	4.7 (2.1)	38.6
Control	4.2	268 (15.9)	87 (9.3)	-	3.8 (1.9)	7.6 (2.7)	-
LSD at 5%	0.81	(5.49)	(1.41)		(0.25)	(0.52)	

* Grades of 1 = 10%, 2 = 11 - 25%, 3 = 26 - 40%, 4 = 41 - 60%, and 5 = > 60% leaf area consumed.

** $\bar{N}$ transformation.

TABLE II

Effect of neem extracts and plumbagin (all at 0.1% concentration) on the survival and development of first-instar larvae of Mythimna separata (Pot assay - Five 1st-instar larvae were confined on treated seedlings for 5 days (five replications per treatment). Leaf disc assay - Ten 1st-instar larvae were confined with the leaf disc for 5 days (five replications per treatment).)

Treatment	Pot assay		Leaf disc assay	
	Larval survival after 5 days (%)	Mean larval weight (mg)	Damage rating*	Larval survival after 5 days (%)
Shade-dried seeds (solid fraction 'G')	48	0.9	1.2 (1.1)**	8
Plumbagin	64	5.6	1.3 (1.3)	32
Shade-dried seeds (alcoholic extract)	72	1.3	2.2 (1.5)	24
Unripe fruits (alcoholic extract)	80	1.4	1.3 (1.1)	14
Shade-dried seeds (petroleum ether extract)	76	4.7	4.1 (2.0)	44
Neem flowers (alcoholic extract)	88	5.3	3.5 (1.9)	30
Control	96	5.8	2.7 (1.6)	32
LSD at 5%		1.44	(0.39)	

* Grades of 1 = 10%, 2 = 11 - 25%, 3 = 26 - 40%, 4 = 41 - 60%, and 5 = > 60% leaf area consumed.

** $\bar{N}$ transformation.

In another experiment (Table III) the activity of five neem extracts was assessed using leaf disc assay and 1st-instar larvae. The acetone-soluble fraction from the unripe fruits was the most active, followed by fraction 'E' of the kernel and the

TABLE III

Effect of five neem extracts (0.1% concentration) on 1st-instar larvae of Mythim na separata (Ten 1st-instar larvae were confined with treated leaf discs for 72 h (five replications per treatment).)

Treatment	Damage rating*	Larval survival (%)	Larval weight (mg)
Hexane ext. of leaves	2.4 (1.5)**	38	4.2 (0.64)
Fraction 'E' of kernel	1.6 (1.2	22	3.9 (0.69)
Fraction 'E' of whole seed	1.6 (1.2)	30	4.2 (0.71)
Acetone-soluble fraction of unripe seeds	1.4 (1.2)	24	4.7 (0.75)
Alcoholic ext. of leaves	2.0 (1.4)	38	3.6 (0.68)
Control, untreated	3.4 (1.8)	70	8.5 (0.91)
Control, chloroform	1.6 (1.2)	28	3.1 (0.61)
Control, acetone	3.8 (1.9)	78	8.3 (0.90)
Control, methanol	2.6 (1.6)		5.7 (0.74)
LSD at 5%	(0.39)		(0.017)

* Grades of 1 = 10%, 2 = 11 - 25%, 3 = 26 - 40%, 4 = 41 - 60%, and 5 = > 60% leaf area consumed

** $\bar{N}$ transformation.

whole seed, and the alcoholic extract of the leaves. Fraction 1, obtained by partitioning the ethanolic extract of the fresh kernels, was the most active and the separated residue insoluble in the solvents also showed phagodeterrent activity, equal to that of fraction 2 (Table IV). However, these fractions were less active than the parent ethanolic extract and fraction 'G', both in terms of leaf feeding and of larval survival.

Effect on development and survival: Larval weight, recorded after feeding on the treated plants in pot assay experiments, was also significantly reduced as compared to the larvae fed on untreated plants. At the end of the test, 3rd-instar larvae averaged only 36 mg in fraction 'G', compared with 87 mg in the control. The 1st-instar larvae had a mean weight of 0.9 mg when fed on leaf discs treated with fraction 'G', and 6 mg in the control. The larval survival was higher in the pot assay than in the leaf disc assay, probably because of the greater leaf surface area and the fresh leaf growth available for the movement and feeding of the larvae in the pot assay (Table II). Fraction 'G' had the maximum effect on larval survival; only 48% of the larvae survived on plants treated with fraction 'G', as compared with 96% in the control (Table II). In another experiment, the larval survival was least on plants treated with fraction 'E' of kernels (22%), followed by the acetone-soluble fraction of the unripe fruits (24%) (Table III). The larval weight was least among the larvae confined to the alcoholic extract of the leaves (3.6 mg), followed by fraction 'E' of the kernel (3.9 mg) and the whole seed (4.2 mg). Only 3% of the larvae reared on the plants treated with fraction 'G' were able to pupate and no adults emerged from these pupae (Fig. 4) compared with 83% adult emergence in the control. The insect took 42

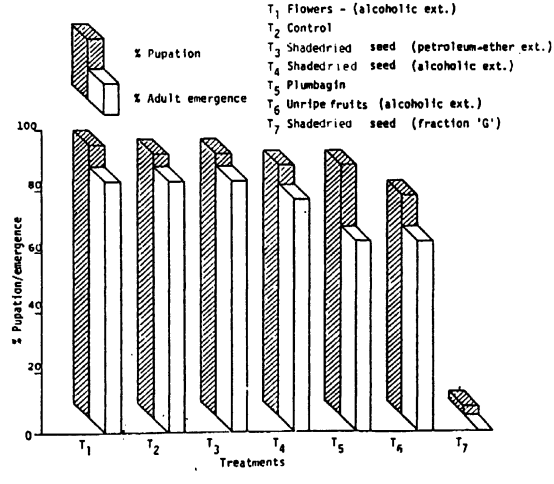

Fig. 4. Effect of five different neem extracts and plumbagin on the pupation and adult emergence of third-instar larvae of *Mythimna separata*.

days to complete its development when fed on the plants treated with the alcoholic extracts of the unripe fruits, as compared with 30 days in the control (Fig. 5).

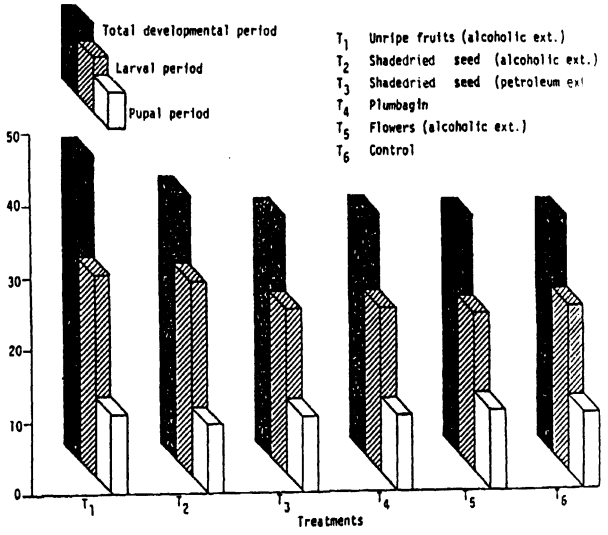

Fig. 5. Effect of four different neem extracts and plumbagin on the development of third-instar larvae of *Mythimna separata*.

Effect on oviposition: Egg laying was delayed in the moths emerging from the treated plants. The delay was maximum with the alcoholic extracts of unripe neem fruits and shade-dried seeds, and considerable with plumbagin (Fig. 6). The total number of eggs laid by the adults from the same number of treated larvae was much lower with the alcoholic extracts of unripe fruits and with plumbagin (Fig. 7). The number of eggs laid per female was the lowest in plumbagin treatments.

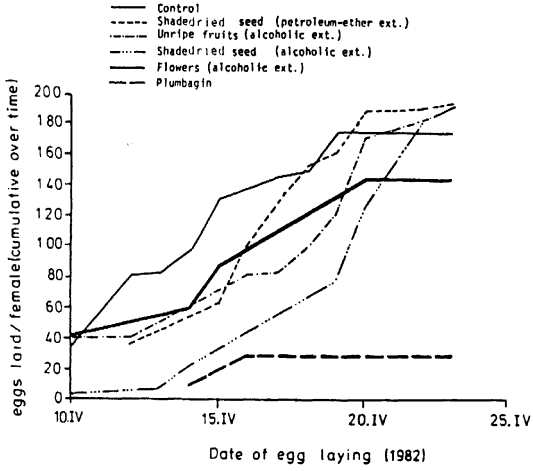

Fig. 6. Number of eggs laid per female moth when third-instar larvae had been exposed to four different neem extracts and plumbagin for 3 days.

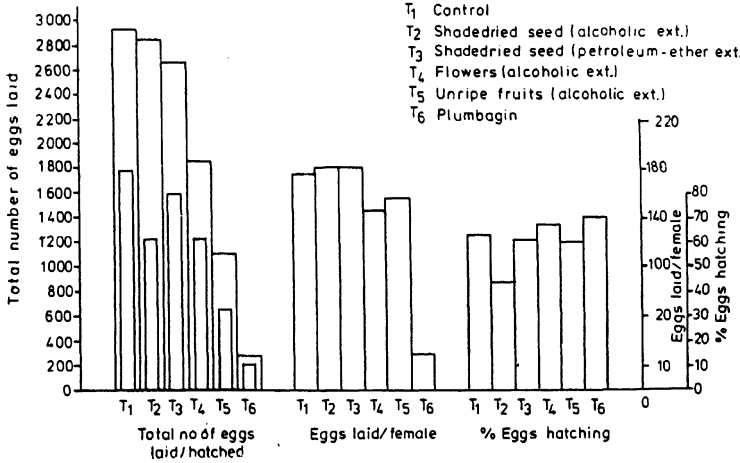

Fig. 7. Oviposition behavior of moths when third-instar larvae had been exposed to five different neem extracts and plumbagin for 3 days.

TABLE IV

Effect of neem extract and its fraction (all at 0.05% concentration) on leaf feeding and survival of third-instar larvae of Mythimna separata.(Two third-instar larvae were confined on treated leaf discs for 48 h (ten replications per treatment).)

Treatment	Damage rating*	Larval survival (%)
Neem kernel, ethanol extract	1.3 (1.1)**	5
Methanol : water (9 : 1)		
Partition 1	1.8 (1.3)	25
2	2.7 (1.6)	30
3	4.3 (2.1)	65
4	4.2 (2.0)	80
5	4.6 (2.1)	70
6	4.6 (2.2)	90
7	4.8 (2.2)	55
Residue of partition 1	2.6 (1.6)	45
Fraction 'G'	1.1 (1.0)	35
Control, acetone	5.0 (2.2)	100
Control, methanol	4.8 (2.2)	95
Control, untreated	5.0 (2.2)	100
LSD at 5%	(0.17)	

* Grades of 1 = 10%, 2 = 11 - 25%, 3 = 26 - 40%, 4 = 41 - 60%, and 5 = > 60% leaf area consumed.

** $\bar{N}$ transformation.

Bioassay of fraction 'G': Having identified 'G' as the most active fraction of the alcoholic extract of the dried neem seeds, further studies were largely confined to the identification of the active components in this extract. The biological properties of fraction 'G' were bioassayed in a range of concentrations, from 0.01 to 0.25% (Table V). The damage rating and larval mortality of the 3rd-instar larvae were proportional to the concentrations. Leaf feeding and larval weight were significantly reduced even at the lower concentration tested (0.01%), and all the larvae died at the highest concentration (0.25%). In the case of 1st-instar larvae, all the larvae died in all the concentrations in a 5-day exposure to the treated plants. The LC_{50} and LC_{95} were found to be 0.023 and 0.14%, respectively.

TABLE V

Effect of different concentrations of fraction 'G' on leaf feeding and mortality of first- and third-instar larvae of Mythimna separata *(pot assay).* (Ten first- or third-instar larvae were confined with the treated plants for 5 days in each pot (three replications per treatment).)

Concentration (%)	First-instar larvae		Third-instar larvae			
	Damage rating*	Mortality (%)	Damage rating*	Unconsumed plant (dry wt., mg)	Mean larval weight (mg)	Mortality (%)
0.01	1	100	2.8	48	22.1	40
0.025	1	100	2.7	63	20.6	50
0.05	1	100	2.3	61	25.4	70
0.1	1	100	1.2	69	16.6	80
0.25	1	100	1.0	52	-	100
0 (control)	4	53	5.0	25	65.0	0
LSD at 5%	0.9	25	0.42	14.8	13.5	29

Grades of 1 = 10%, 2 = 11 - 25%, 3 = 26 - 40%, 4 = 41 - 60%, and 5 = > 60% leaf area consumed.

Biological activity of the chromatographic fractions

Effect of feeding: Fractions AI-1 to AI-14 and extracts 'G' and 'M' were bioassayed for their biological activity at 0.05% concentration, using the leaf disc assay with 1st-instar larvae (Table VI). The larvae were confined to the treated leaf discs for 72 h. Of the various fractions obtained, AI-9, AI-10, and AI-11 exhibited a phagodeterrent effect. Fractions AI-10 and AI-11 were each as active as fraction 'G'. The biological activity of the active fractions AI-9, AI-10 and AI-11, along with the parent fractions 'G' and 'M', were further tested at 0.01 and 0.05% concentrations, using 1st- and 3rd-instar larvae. The extent of leaf damage was least in fractions AI-10 and 'G' (Tables VII, VIII).

TABLE VI

Effect of several neem fractions (all at 0.05% concentration) on leaf feeding and development of first-instar larvae of Mythimna separata (Ten first-instar larvae were confined with the treated leaf discs for 72 h (three replications per treatment).)

Fraction	Damage rating*	Unconsumed leaf disc (dry wt., mg)	Larval wt. (mg) (72 h after confinement)	Larval mortality (%)	Larvae pupated (%)	Adults emerged (%)	No. of females	Eggs laid/ female	Larvae hatched/ female	Larval hatching (%)
'G'	1.3	28.3 (5.3)**	4.7 (2.2)	100	00	-	-	-	-	-
'A'	1.5	32.1 (5.7)	4.2 (2.0)	100	00	-	-	-	-	-
AI-9	1.8	24.4 (4.9)	7.5 (2.5)	100	00	-	-	-	-	-
AI-10	1.3	24.8 (5.0)	5.0 (2.1)	100	00	-	-	-	-	-
AI-11	1.3	26.3 (5.1)	6.8 (2.3)	100	0	-	-	-	-	-
AI-12	4.5	19.9 (4.5)	21.2 (4.6)	63	37	17	2	72	0	0
AI-7	4.0	20.6 (4.5)	30.6 (5.4)	67	33	33	5	-	-	-
AI-8	4.0	23.7 (4.8)	19.1 (4.4)	77	23	17	4	175	0	0
AI-14	4.0	22.0 (4.7)	19.5 (4.4)	53	47	33	3	294	170	83
AI-1	5.0	25.3 (5.0)	20.1 (4.5)	40	60	40	6	536	335	63
AI-3	4.7	18.0 (4.2)	24.6 (4.9)	30	70	57	4	94	77	82
AI-4	4.8	20.9 (4.6)	9.9 (3.4)	40	60	43	6	179	133	78
AI-5	4.3	25.2 (5.0)	22.5 (4.7)	40	60	33	6	588	367	62
AI-6	4.7	30.0 (5.5)	23.6 (4.8)	42	57	37	4	717	445	62
AI-13	4.5	26.1 (5.1)	23.4 (4.8)	30	70	33	4	-	-	-
Control, acetone	4.7	18.2 (4.3)	18.5 (4.3)	40	60	57	9	793	592	75
Control, methanol	4.7	16.3 (4.02)	27.6 (5.2)	43	57	30	4	646	547	85
LSD at 5%	0.26	(0.58)	(1.39)							

* Grades of 1 = 10%, 2 = 11 - 25%, 3 = 26 - 40%, 4 = 41 - 60%, and 5 = > 60% leaf area consumed.

** $\bar{N}$ transformation.

TABLE VII

Effect of five neem fractions (all at 0.01% concentration) on the leaf feeding and survival of first-instar larvae of Mythimna separata. (Ten first-instar larvae were confined on treated leaf discs for 72 h (five replications per treatment).)

Fraction	Damage rating*	Larval weight (mg)	Larval survival (%)
'G'	1.9 (1.3)**	0.43 (0.7)	0
'M'	2.9 (1.7)	0.44 (0.7)	6
AI-9	3.1 (1.7)	0.39 (0.5)	0
AI-10	2.6 (1.8)	0.40 (0.7)	0
AI-11	3.5 (1.9)	0.34 (0.5)	0
Control, acetone	5.0 (2.2)	0.70 (0.8)	66
Control, untreated	3.9 (2.0)	1.30 (1.1)	64

* Grades of 1 = 10%, 2 = 11 - 25%, 3 = 26 - 40%, 4 = 41 - 60%, and 5 = > 60% leaf are consumed.

** $\bar{N}$ transformation.

TABLE VIII

Effect of five neem fractions (all at 0.05% concentration) on the feeding and survival of third-instar larvae of Mythimna separata. (Five third-instar larvae were confined on treated leaf discs for 48 h (five replications per treatment).)

Fraction	Damage rating*	Leaf area consumed (cm^2)	Larval weight (mg)	Larval mortality (%)	Larval pupated (%)	Moths emerged (%)
'G'	1.2 (1.1)**	12.2 (3.5)	6.7 (2.6)	76	24	16
'M'	2.2 (1.5)	10.5 (3.2)	9.4 (3.0)	76	24	8
AI-9	1.4 (1.2)	11.5 (3.4)	7.4 (2.7)	76	24	0
AI-10	1.3 (1.1)	12.0 (3.5)	6.4 (2.5)	80	20	0
AI-11	1.4 (1.2)	11.9 (3.4)	6.6 (2.5)	76	24	8
Control, acetone	4.3 (2.1)	6.8 (2.6)	17.4 (4.1)	36	64	40
Control, untreated	3.5 (1.7)	7.1 (2.6)	16.0 (4.0)	36	64	56
LSD at %%	(0.32)	(0.48)	(0.60)			

* Grades of 1 = 10%, 2 = 11 - 25%, 3 = 26 - 40%, 4 = 41 - 60%, and 5 = > 60% leaf area consumed.

** $\bar{N}$ transformation

Effect on development and survival: None of the larvae was able to pupate when fed on leaf discs treated with fraction 'G', 'M', AI-9, AI-10 or AI-11 (Table VI). Although fractions AI-8 and AI-12 did not show any phagodeterrency or reduce larval weight, they did reduce the percent pupation and adult emergence significantly. Larval mortality occurred earlier with fractions AI-10 and AI-11, and all the larvae died by the 5th day (Fig. 8). This was followed by fractions AI-9, 'M' and 'G', in which mortality reached 100% in 15 days. The activity of fractions AI-9, AI-10 and AI-11 was higher than the parent extracts 'G' and 'M', in terms of larval mortality.

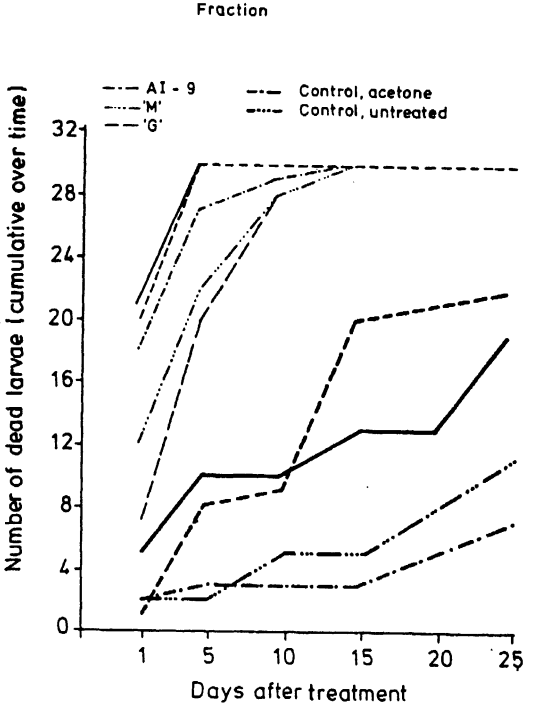

Fig. 8. Effect of two extracts and five different active neem fractions on the mortality of first-instar larvae of *Mythimna separata*.

Effect on oviposition: The number of eggs laid per female was substantially lower (< 200) in females emerging from the larvae fed on leaf discs treated with fractions AI-3, AI-4, AI-8 and AI-12, as compared to 646 eggs recorded in the untreated control. The reasons for reduced egg laying are not clear. Egg hatch varied from 62 to 85%, and the biological significance of this needs to be studied further (Table VI).

Larval weight was significantly reduced in fractions 'G', 'M', AI-9 and AI-11 (Tables VI, VII, VIII). None of the first instar larvae was able to pupate when fed on leaf discs treated with a 0.01% concentration of different neem fractions (Table VII). No adults emerged in the 0.05% treatments with fractions AI-9 and AI-10, compared with 56% emergence in the untreated control (Table VIII).

Mortality of the 1st- and 3rd-instar larvae occurred earliest following with fraction AI-10 (Fig. 9). By the 8th day after confinement, the larval mortality in fractions AI-9, AI-10 and AI-11 was higher than in the parent extracts 'G' and 'M'.

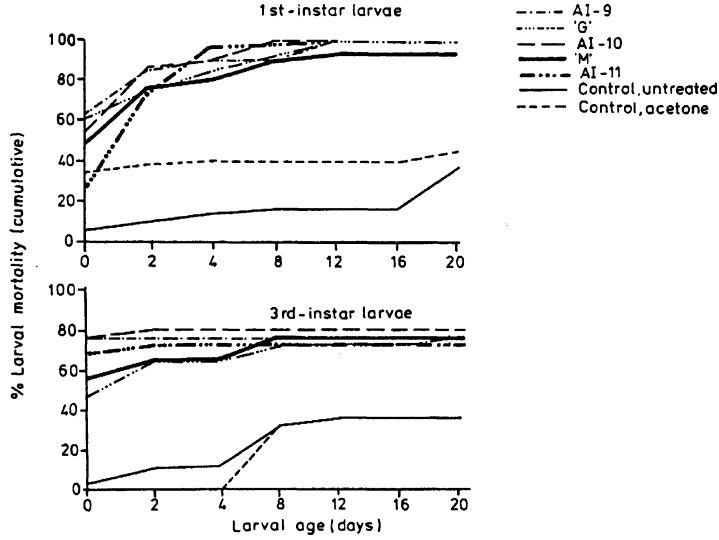

Fig. 9. Effect of two extracts and three different active neem fractions on the mortality of first- and third-instar larvae of *Mythimna separata*.

Effect on molting: At the end of 72 h of feeding on leaf discs treated with different extracts, the larvae were observed under a microscope as to the effects on the molting process. The observations recorded are described below:

In extract 'G' treatments the larvae were unable to shed the old head capsule during the molting process. Larvae were small in size and black fluid had accumulated in the thorax. Most of the larvae were in the molting process in fraction 'M' treatments and they seemed to be unable to emerge from the old integument. The larvae were light gray in color instead of the normal green yellow. Some larvae had red colored bands in the pleural region.

In fraction AI-9 treatments most of the larvae were in the molting process. One larva died because of difficulty in molting and its integument had become pink in color. Some larvae died in the fraction AI-10 treatments and others were unable to molt. Most of the dead or dying larvae were reddish gray in color. In fraction AI-11 treatments most of the dying larvae were light red in color.

Larvae were in the molting process in the acetone-treated and untreated controls. The molting larvae had a distinct suture in the old integument at the thoracic region. The larvae were light green in color.

Biological activity of fraction 'G' under field conditions

Field tests of fraction 'G' from kernels against *M. separata* showed that the extent of leaf damage in the neem-treated plot of pearl millet hybrid BJ-104 was substantially less than in the control or the malathion-treated plots, and greater than in the fenvalerate-treated plot (Fig. 10). The extent of damage in the buffer plots around the neem-trated plot was greater than in the buffer plots around the control plot, indicating some movement of larvae out of the neem-treated plot.

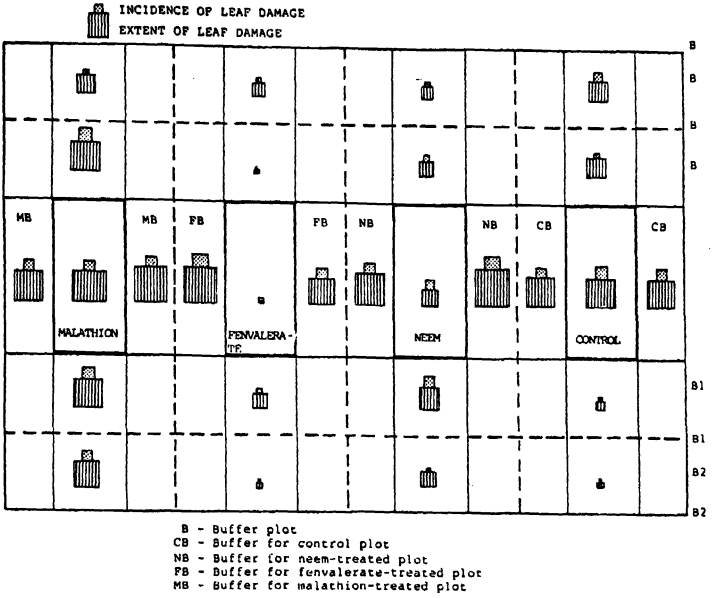

Fig. 10. Schematic drawing of the incidence and extent of leaf damage to pearl millet plants by larvae of *Mythimna separata*.

Fraction 'G' did not affect oviposition or dead heart formation due to the sorghum shootfly (Table IX). The extent and incidence of armyworm damage due to the inoculated larvae were much lower in the neem-sprayed plot (damage rating = 1, incidence = 50.5%) as compared with the control plots (damage rating = 3, incidence = 79.9%). The numbers of maize aphids, *Rhopalosiphum maidis*, in the neem-sprayed plot was generally higher than in the control plot, while the number of shootbugs, *Peregrinus maidis*, tended to be lower in the neem-treated plot. The population of headbugs, *Calocoris angustatus*, was lower in the neem-treated plots, and there was a slight reduction in the numbers of midges, *Contarinia sorghicola*, and thrips, *Thrips* sp. The midge damage in the peak activity period was not influenced by neem sprays, while the 1000-grain weight (influenced by headbug feeding) rose by nearly 2/1000 grains. Of the 100 larvae of *Mythimna separata*

TABLE IX

Effect of neem spray on insect pests of sorghum in the field (A. s. = *Atherigona soccata*; R. m. = *Rhopalosiphum maidis*; M. s. = *Mythimna separata*; P. m. = *Peregrinus maidis*; C. a. = *Calocoris angustatus*; C. s. = *Contarinia sorghicola*)

	Date of observation 1983	Number of plants or heads observed		Neem treatment	Control
Seedling and foliage feeders	28. I.	406	A. s., dead hearts (%)	34.6	36.9
		406	A. s. eggs/100 plants	55.7	52.9
	4. II.	650	A. s., dead hearts (%)	39.9	41.5
		40	A. s. eggs/100 plants	30.6	29.9
			No. of R. m./10 plants	27.0	5.0
			Damage rating*	1	3
	11. II	424	M. s. damage (%)	30.5	79.9
		424	No. of M. s. larvae/100 plants	61.9	176.8
		40	No. of R. m./10 plants	106.3	49.5
		40	No. of P. m./10 plants	19.5	30.5
			Damage rating*	1	4
	18. II.	250	M. s. damage (%)	49.3	95.4
		250	No. of M. s. larvae/100 plants	6.5	13.6
		40	No. of R. m./10 plants	49.0	22.0
		40	No. of P. m./10 plants	11.5	16.0
			Damage rating*	1	4
	25. II.	330/plot	M. s. damage (%)	69.7	92.4
		40	No. of R. m./10 plants	247.0	132.0
		40	No. of P. m./10 plants	12.0	25.0
Earhead feeders	4. III.	40	No. of C. a./10 heads	61	100
		40	No. of C. s./10 plants	4	8
		40	No. of thrips/10 heads	50	59
	14. III.	40	No. of C. a./10 heads	90	188
		40	No. of C. s./10 heads	12	10
		40	No. of thrips/10 heads	32	40
	6. III - 6. IV.	1100	No. of C. s. flies/100 heads	132	172
At harvest	22. IV.	2000 florets	C. s. damage (%)**	49.4	49.4
			Top-anthesis	49.4	49.4
			Post-anthesis	32.7	33.9
			Milky	30.8	32.1
		4000 grains	C. a. damage, 1000-grain wt (g)*		
			Top-anthesis	28.25	27.62
			Post-anthesis	34.23	32.19
			Milky	35.33	33.77

* Grades of 1 = 10%, 2 = 11 - 25%, 3 = 26 - 40%, 4 = 41 - 60%, and 5 = > 60% leaf area consumed.

** Heads tagged on 14. III. 1'83, at different developmental stages.

collected from the neem-treated and control plots (Table X), only three adults emerged from the neem-treated plot, as against 62 from the control. The sprays resulted in a 25% increase in head weight and a 30% increase in grain yield over the control (Table XI).

TABLE X

Effect of one neem spray (extract 'G', 0.1%) on pupation and adult emergence of Mythimna separata *larvae released in a sorghum field.* (Larvae collected one week after spraying.)

Observation	Neem spray	Control
Number of larvae collected	100	100
Number of larvae pupated	21	83
Number of moths emerged	3	62

TABLE XI

Effect of seven weekly sprays of neem extract 'G' (0.1%) on head weight and grain yield of sorghum

		Weight (kg) per 51 m^2 plot		% Increase of treatment over control
		Neem	Control	
Head weight	$\overline{X}$	13.8 (11.0 - 17.0)	11.0 (10.0 - 13.0)	25
Grain yield	$\overline{X}$	10.4 (8.5 - 13.0)	8.0 7.0 - 9.0	30

$\overline{X}$ = Mean of four subsamples and range (in parantheses).

Observations on the effect of the neem extract on natural enemies showed that the larval parasitization (mostly by *Apanteles ruficrus*) of *M. separata* was reduced, while the midge parasitization by *Tetrastichus* sp. was slightly higher in the neem-treated plot than in the control of (Table XII). There did not appear to be much effect on the activity of the midge predator, *Orius* sp.

TABLE XII

Effect of neem spray (extract 'G', 0.1%) on parasites and predators of Mythimna separata *and* Contarinia sorghicola

Insect	Parasite or predator	Sample size	Neem	Control
M. separata	*Apanteles ruficrus*	% Parasitization on 100 larvae	6	13
C. sorghicola	*Orius* sp.	No. of predators per 10 heads		
		on 4. III.	98	117
		14. III.	93	92
	Tetrastichus sp.	% Parasitization on 400 florets		
		Top-anthesis	15	12
		Post-anthesis	19	15

DISCUSSION

The results of the phagodeterrency assays using pot and leaf disc techniques with 1st- or 3rd-instar larvae of *Mythimna separata*, were similar. The leaf disc assay technique used is quite easy, efficient and rapid, and allows for the handling of a large number of fractions at the same time. Among the different neem extracts, the solid fraction 'G' obtained from the ethanolic extract of shade-dried seeds was the most effective against the 1st- and 3rd-instar larvae of *M. separata*. The potential activity of fraction 'G' can be seen from its effects on larval weight, pupation and adult emergence. No adults emerged from the larvae which fed on 12 plants treated with 0.1% fraction 'G'. The alcoholic extract of the unripe fruits also showed high biological activity, as measured by its adverse effects on larval weight, pupation and adult emergence, and the prolonged developmental period of the exposed larvae. The potential activity of the unripe fruits was also evident from the activity of the acetone-soluble components. Extracts of the seed or the seed kernel are known to be the most active (Goyal *et al.*, 1971. However, the alcoholic extract of the leaves also showed an adverse effect on the larval weight. This could probably be due to its growth-disrupting effects (Leuschner, 1972).

Fraction 'G' showed phagodeterrent and toxic effects even at the low concentration of 0.01%, against larvae of *M. separata;* its LC_{50} against the 3rd-instar lar-

vae was 0.023%, which is quite similar to that of some currently used insecticides. There is more than one chemically active compound in neem kernels, as evidenced by column chromatography and partitioning of the crude extracts. The three active fractions (AI-9, AI-10, AI-11) obtained from fraction 'M' were each as phagodeterrent as was the parent extract. However, larval mortality in treatments with these fractions occurred much earlier than in the parent fractions 'G' and 'M'. Fraction AI-10 was the most active. Although, fractions AI-8 and AI-12 resulted in larval mortality and reduced egg laying, they did not show any antifeedant activity. A similar action of some fractions was reported by Schmutterer & Rembold (1980). The phagodeterrent/repellent properties of neem extracts have been confirmed by various workers with insects belonging to such diverse orders as Orthoptera, Hemiptera, Coleoptera, Diptera and Lepidoptera (Schmutterer, 1981). The results of experiments with pure azadirachtin are known to be on a par with the crude extracts (Schmutterer, 1981). The present investigations have also shown that the different column chromatographic fractions are as active or more so than the parent fraction 'G'.

The growth-disrupting effects of the crude extracts as well as the pure fractions add to the usefulness of neem in practical pest control. The inability of the armyworm larvae to emerge from its old integument, accumulation of black fluid in the thoracic region, and the development of a reddish gray color in the larvae suggest some interaction between the neem components and the molting processes of the larvae. Neem components have been reported to inhibit and disrupt the development of a number of insect species (McMillan *et al.*, 1969; Leuschner, 1972; Ruscoe, 1972; Meisner *et al.*, 1976, 1978; Redfern *et al.*, 1979; Sharma *et al.*, 1980; Rembold *et al.*, 1980; Schmutterer & Rembold, 1980; Schmutterer, 1981; Ascher & Gsell, 1981; Lange & Schmutterer, 1982; Steffens & Schmutterer, 1982).

Another important effect of the kernel extracts was the delayed egg laying in the adults from larvae fed on plants treated with alcoholic extracts of unripe fruits and shade-dried seeds. There were slight differences in the number of eggs laid per female and in larval hatching. Detailed studies are in progress to determine the biological significance of the observed differences. Reduced fecundity and egg hatching have been reported in a number of insects (Steets & Schmutterer, 1975; Jacobson *et al.*, 1978; Rembold & Sieber, 1981; Schulz, 1981).

The effectiveness of fraction 'G' against the 3rd-instar larvae of *M. separata* was also confirmed under field conditions, with sorghum and pearl millet. Neem sprays reduced the insect population and damage caused by shootbugs, headbugs and, to some extent, thrips. However, there was no apparent effect on shootfly,

aphids and midges. This was probably because of differences in the feeding behavior of these insects. The shootfly maggot feeds on the growing point inside the stem, while the aphid and the midge maggot suck the sap from leaf tissues and ovary, respectively. The midge larva also remains protected inside the glumes.

The reduced parasitism by *Apanteles ruficrus* of the *M. separata* larvae in the field was probably a result of the premature larval mortality, or perhaps the neem extracts are repellent to the parasites. Similar reduced parasitism of larvae as a result of neem application has been reported in the rice leaf folder (Saxena *et al.*, 1981). There was no affect of neem sprays on the midge parasite (*Tetrastichus* sp.) or on the midge predator (*Orius* sp.).

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REFERENCES

ASCHER, K.R.S. & GSELL, R. (1981). The effect of neem seed kernel extract on *Epilachna varivestis* Muls. larvae. *J. Pl. Dis. Prot.* **88** : 764 - 767.

BUTTERWORTH, J.H. & MORGAN, E.D. (1968). Isolation of a substance that suppresses feeding in locusts. *Chem. Comm.* 23 - 24.

GOYAL, R.S., GULATI, K.C., SARUP, P., KIDWAI, M.A. & SINGH, D.S. (1971). Biological activity of various alcoholic extractives and isolates of neem *(Azadirachta indica)* seed cake against *Rhopalosiphum nymphaeae* (Linn.) and *Schistocerca gregaria* Forsk. *Indian J. Ent.* **33** : 67 - 71.

JACOBSON, M., REED, D.K., CRYSTAL, M.M., MORENO, D.S. & SODERSTROM, E.L.C. (1978). Chemistry and biological activity of insect feeding deterrents from certain weed and crop plants. *Entomologia exp. appl.* 24 : 248 - 257.

JOSHI, B.G. & RAMAPRASAD, G. (1979). Neem kernel as an antifeedant against the tobacco caterpillar (*Spodoptera litura* F.). *Phytoparasitica* 3 : 59 - 61.

—— & SITARAMAIAH, S. (1979). Neem kernel as an ovipositional repellent for *Spodoptera litura* F. moths. *Phytoparasitica* 7 : 199 - 202.

KRAUS, W., CRAMER, R., BOKEL, M. & SAWITZKI, G. (1981). New insect antifeedants from *Azadirachta indica* and *Melia azedarach*. *Proc. 1st Int. Neem Conf.* (Rottach-Egern, 1980), pp. 53 - 62.

LADD, T.L. Jr., JACOBSON, M. & BURIFF, C.R. (1978). Japanese beetles: Extracts from neem tree seeds as feeding deterrents. *J. econ. Ent.* 71 : 810 - 813.

LANGE, W. & SCHMUTTERER, H. (1982). Experiments with synergists to improve the effect of growth disrupting properties of a methanolic extract of seeds of the neem tree *(Azadirachta indica)*. *J. Pl. Dis. Prot.* 89 : 258 - 265.

LAVIE, D., JAIN, M.K. & SHPAN-GABRIELITH, S.R. (1967). A locust phagorepellent from two *Melia* species. *Chem. Comm.* 910 - 911.

LEUSCHNER, K. (1972). Effect of an unknown plant substance on a shield bug. *Naturwissenschaften* 59 : 217 - 218.

McMILLAN, W.W., BOWMAN, M.C., BURTON, R.L., STARKS, K.J. & WISEMAN, B.R. (1969). Extract of chinaberry leaf as a feeding deterrent and growth retardant for the larvae of the corn-earworm and fall armyworm. *J. econ. Ent.* 62 : 798 - 710.

MEISNER, J., KEHAT, M., ZUR, M.A. & EIZICK, C. (1978). Response of *Earias insulana* Boisd. larvae to neem (*Azadirachta indica* A. Juss) kernel extract. *Phytoparasitica* 6 : 85 - 88.

—— , WYSOKI, M. & ASCHER, K.R.S. (1976). The residual effect of some products from neem (*Azadirachta indica* A. Juss) seeds upon larvae of *Boarmia Ascotis) selenaria* Schiff. in laboratory trials. *Phytoparasitica* 4 : 185 - 192.

MORGAN, E.D. (1981). Strategy in the isolation of insect control substances from plants. *Proc. 1st Int. Neem Conf.* (Rottach-Egern, 1980), pp. 43 - 52.

NAKANISHI, K. (1975). Structure of the insect antifeedant azadirachtin. *In:* RUNECKLES, V.C. IEd.I *Recent Advances in Phytochemistry*. Plenum, New York, NY, Vol. 5, pp. 283 - 298.

PRADHAN, S., JOTWANI, M.G. & RAI, B.K. (1962). The neem seed deterrent to locust. *Indian Fmg* 12 : 7 - 11.

PRUTHI, H.S. (1937). Report of the Imperial Entomologist. *Sci. Re. Agric. Res. Inst., New Delhi,* 1935 - 36.

REDFERN, R.E., WARTHEN, J.D. Jr., MILLS, G.D Jr. & UEBEL, E.C. (1979). Molting inhibitory effects of azadirachtin on large milk weed bug. *Dep. Agric., Agric. Res. Results, ARR-NE-5.*

REMBOLD, H. , SHARMA, G.K., CZOPPELT, Ch. & SCHMUTTERER, H. (1980). Evidence of growth disruption in insects without feeding inhibition by neem seed fractions. *Z. PflKrankh. PflSchutz* **87** : 291 - 297.

—— & SIEBER, K.-P. (1981). Effect of azadirachtin on oocyte development in *Locusta migratoria migratorioides. Proc. 1st Int. Neem Conf.* (Rottach-Egern, 1980), pp. 75 - 80.

RUSCOE, C.N.E. (1972). Growth disruption effects of an insect antifeedant. *Nature, New Biol.* **236** : 159 - 160.

SAXENA, R.C., WALDBAUER, G.P., LIQUIDO, N.J. & PUMA, B.C. (1981). Effects of neem seed oil on the rice leaf folder, *Cnaphalocrocis medinalis. Proc. 1st Int. Neem Conf.* (Rottach-Egern, 1980), pp. 189 - 204.

SCHMUTTERER, H. (1981). Some properties of components of the neem tree *(Azadirachta indica)* and their use in pest control in developing countries. *Meded. Fac. Landbouww. Rijksuniv. Gent* **46** : 39 - 47.

—— , ASCHER, K.R.S. & REMBOLD, H. (Edsl (1981). *Natural pesticides from the neem tree (Azadirachta indica A. Juss). Proc. 1st Int. Neem Conf.* (Rottach-Egern, 1980), 297 pp.

—— & REMBOLD, H. (1980). Performance of some poor fractions extracted from the seeds of *Azadirachta indica* on feeding activity and metamorphosis of *Epilachna varivestis* (Col., Coccinellidae). *Z. angew. Ent.* **89** : 179 - 188.

SCHULZ, W.-D. (1981). Pathological alterations in the ovaries of *Epilachna varivestis* induced by an extract from neem kernels. *Proc. 1st Int. Neem Conf.* (Rottach-Egern, 1980), pp. 81 - 96.

SHARMA, G.K., CZOPPELT, Ch. & REMBOLD, H. (1980). Further evidence of growth disruption by neem seed fractions. *Z. angew. Ent.* **90** : 439 - 444.

SHARMA, H.C., BHATNAGAR, V.S. & DAVIES, J.C. (1982). Studies on *Mythimna separata* at ICRISAT. *Sorghum Entomology Progress Report - 6. International Crops Research Institute for the Semi-Arid Tropics,* Patancheru, India.

STEETS, R. & SCHMUTTERER, H. (1975). Einfluß von Azadirachtin auf die Lebensdauer und das Reproduktionsvermögen von *Epilachna varivestis* Muls. (Coleoptera, Coccinellidae). *Z. PflKrankh. PflSchutz* **82** : 176 - 179.

STEFFENS, R.J. & SCHMUTTERER, H. (1982). The effect of a crude methanolic neem *(Azadirachta indica)* seed kernel extract on metamorphosis and quality of adults of the Mediterranean fruit fly, *Ceratitis capitata* Wied. (Diptera, Tephritidae). *Z. angew. Ent.* **94** : 98 - 103.

STEFFENS, R.J. & SCHMUTTERER, H. (1982). The effect of a crude methanolic neem *(Azadirachta indica)* seed kernel extract on metamorphosis and quality of adults of the Mediterranean fruit fly, *Ceratitis capitata*, Wied. (Diptera, Tephritidae). *Z. angew. Ent.* **94** : 98 - 103.

WARTHEN, J.D. Jr., REDFERN, R.E., UEBEL, E.C. & MILLS, G.D. Jr. (1978a). An antifeedant for all armyworm larvae from neem seeds. *U.S. Dep. Agric. Agric. Res. Results, ARR-NE-1.*

——, UEBEL, E.C., DUTKY, S.R., LUSBY, W.R. & FINEGOLD, H. (1978b). Adult housefly feeding deterrent from neem seeds. *U.S. Dep. Agric., Agric. Res. Results, ARR-NE-2.*