J. ent. Res., 30 (3) : 225-229 (2006)

Effect of *Madhuca indica* seed extracts on survival, feeding and development of *Helicoverpa armigera* (Hubner)

J. Loganathan, Swaran Dhingra, Suresh Walia[1] and K. Shankar Ganesh

Division of Entomology, Indian Agricultural Research Institute, New Delhi - 110 012, India

ABSTRACT

The laboratory study on various extracts of *Madhuca indica* on feeding and development of *Helicoverpa armigera* revealed that the methanol extract, found to be more effective in causing antifeedancy with AI 50 value of 7.14%. The hexane, aqueous extract and saponin at the highest concentration showed less than 50.0% antifeedancy. Methanol exrtract was more effective in reducing the larval weight gain against *H. armigera,* GI 50 value being 5.40% followed by hexane extract 9.32%. When larvae of *H. armigera* were fed with various extracts of *M. indica*, saponin inhibibited normal adult emergence by 50.0% at 0.75% followed by methanol 9.80%, and hexane extract 9.83%.

The noctuid *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) is one of the most important constraint to crop production in Asia, Africa, Australia and Mediterranean Europe. It is a polyphagous pest and attacks over 200 crop species, belonging to 45 families (Sharma, 2001). Globally, this pest causes yield loss worth about US$ 2 billion annually (ICRISAT, 2003). In India, the annual loss due to this pest on pigeon pea and chickpea was estimated as 200 million US dollars (Jackson *et al*. 1989). *H. armigera* control is currently based on heavy use of many neurotoxic insecticides, which are damaging to the environment and/or pose a threat to public health via food residues, ground water contamination, or accidental exposure. The problems caused by pesticides and their residues have amplified the need for effective, biodegradable pesticides with greater selectivity. Alternative strategies have included the investigation for new type of insecticides, and the re-evaluation and use of traditional botanical pest control agents.

Pesticides derived from plants have the potential to play a major role in pest management in sustainable agriculture production. They are renewable, non- persistent in the environment, and relatively safe to natural enemies, non-target organisms, and human beings. Plants produce a range of chemical substances to protect themselves from insect pests. Such chemicals are secondary metabolites and include alkaloids, terpenoids, flavonoids and acetogenins (Parmar and Singh, 1993). Over 2,000 plants species have been reported to possess biological activity against different type of insects. Amongst these, neem (*Azadirachta indica* A Juss.) has been the focus of a large number of studies over the past four decades. Apart from neem, it is wise to develop and have array of botanicals. In the line of development, *Madhuca indica* Vent. have been tried under field condition to reduce pest population (Rajasekaran *et al*., 1987, Mariappan *et al.,* 1988 and Jothi *et al*., 1990). Since there is no detailed investigation about the efficacy of various extracts of *M. indica*, the present study was carried out to compare the various extracts of the plant for their activity.

MATERIALS AND METHODS

Dried pods of *Madhuca indica*, were procured from the market in September at New Delhi. The pods were broken and seeds were removed and dried for 4 days. The kernal obtained was ground to powder by an electric grinder. The powdered Mahua Seed Kernal (MSK) thus obtained was used for extraction with various solvents. For hexane extract,1 kg seed was soaked with 1.5 l. of hexane in a glass jar for 15 minutes and subsequently stirred continuously with the help of mechanical stirrer for 45 minutes to extract oil from the powder. Another

[1] Division of Agricultural Chemicals, Indian Agricultural Research Institute, New Delhi - 110 012, India

45 minutes was allowed to stand and filtered through Whatman no. 1 filter paper in Buchner funnel under vacuum. The cake was again extracted with hexane three times in the same way as explained above. The extract, so obtained was freed of hexane on a rotary flash evaporator at 50^{0}C under reduced pressure. Mahua oil (326.1g) so obtained contained significant amount of saponin. The residue left after oil extraction was dried under fan and collected as deoiled Mahua seed cake, which was used for further extraction.

To obtain Methanol extract, the de-oiled MSK was extracted with 1.0L. of methanol as described above by replacing hexane with methanol. The methanol extract (21.2g) was freed from methanol in a rotary flash evaporator at 45º C under reduced pressure.

To get aqueous extract, 500 g of powdered MSK was stirred with 1000 ml of distilled water using mechanical stirrer. After stirring for one hour, the material was filtered through Whatman no.1 filter paper in a Buchner funnel under vacuum. The powder was once again stirred with 750 ml of distilled water for 45 minutes and filtered. The filtered extracts were combined and removed water at 60 º C using rotary evaporator and obtained 18.2g of conc.aqueous extract.Methanol extract obtained was concentrated to one-third of its volume and the resultant extract partitioned with butanol-water (1:1). The organic layer was concentrated under vacuum at 50°C to remove butanol and the resultant concentrate precipitated with excess of acetone to obtain saponin concentrate. The precipitated saponin (2.02g) were filtered quickly and preserved for future bioassay work.

The various extracts of *M. indica* were diluted with the required quantity of distilled water containing emulsifier (0.5 g Triton X–100 per 100 ml of distilled water) to get 20% stock solution emulsion. The emulsion was further diluted with blank emulsion (5 ml of respective solvent for each extract + 0.5 g Triton X – 100 made to 100 ml of distilled water) for the preparation of final concentrations. This procedure enabled to maintain the solvent and emulsifier level at 5 and 0.5 percent, respectively in the final test concentration.

Insects: Laboratory culture of the gram pod borer, *H.armigera* used in these studies were obtained from an incessant colony developed in an insectary having controlled environment. The conditions were 27 ± 1°C temperature and 70 ± 5% RH, a photophase of 14 hrs, and 10 hrs scotophase.

Antifeedant and IGR bioassay: The third instar larvae of *H. armigera* weighing 30-40 mg were exposed to botanical extracts on cabbage leaves. Leaf discs of approximately 4 cm dia. were cut from cabbage leaves. After washing the leaf discs thoroughly, they were dipped in the required concentration of the extract for twenty seconds and then air-dried. The treated discs were then transferred individually to clean petri plate (8 cm x 1.5 cm) and one 7 ± 1day old larvae were placed in each petri plate. Each treatment and control was replicated twenty times. Observations on the amount of leaf area consumed were recorded at 48 hours after treatment. The percent protection over control was calculated using the following Abbot's modified formula:

$$\text{Per cent leaf protection} = \frac{\text{Leaf area given (cm}^2\text{)} - \text{Leaf area consumed (cm}^2\text{)}}{\text{Leaf area given (cm}^2\text{)}} \times 100$$

$$\text{Per cent antifeedance} = \frac{\text{Per cent leaf protection in treatment} - \text{Per cent leaf protection in control}}{100 - \text{Percent leaf protection in control}} \times 100$$

Following the observations on antifeedance, treated leaf discs were replaced with fresh discs. The development of the treated larvae was monitored up to adult emergence. Data were recorded on per cent larval weight reduction at 3 and 7 days after treatment (DAT) , larval mortality, larval – pupal intermediates (LPI) , pupal weight reduction , pupal mortality, and normal adult emergence. The data was subjected to probit analysis for the calsulation of antifeedance index (AI_{50}), growth inhibition index (GI_{50}), median lethal concentration (LC_{50}) and inhibition of normal adult emergence (I_{50}) values. The percent growth reduction of larvae and pupae over control was computed as follows:

$$\text{\% Reduction in Larval/Pupal wt} = \frac{\text{Larval/Pupal wt. gain in Control} - \text{Larval/Pupal wt. gain in Treatment}}{\text{Larval/Pupal wt gain in Control}} \times 100$$

Table 1. Effect of feeding on various extracts of *M. indica* on biological parameters in *H. armigera*

Treatment	Concentration (%)	% antifeedancy	Larval mortality (%)	Larva		Pupa			Adult	
				Larval weight reduction (%)		% L–P intermediates	% pupal mortality	% pupal weight reduction	% normal adults	% malformed adults
				3 DAT	7 DAT					
	5.0	30.1 (33.3)^{b}	6.7 (12.6)^{b}	46.6 (43.0)^{b}	21.2 (27.3)^{b}	6.7 (15.0)^{b}	20.0 (26.6)^{a}	18.5 (25.4)^{b}	59.9 (50.7)^{b}	6.7 (15.0)^{a}
	3.0	28.7 (32.4)^{b}	6.7 (12.6)^{b}	36.8 (37.3)^{c}	18.4 (25.3)^{bc}	6.7 (15.0)^{b}	13.3 (21.4)^{b}	16.5 (23.9)^{b}	73.3 (58.9)^{c}	0.0 (0.9)^{b}
Hexane	1.0	23.5 (29.0)^{c}	6.7 (12.6)^{b}	36.4 (37.1)^{c}	14.3 (22.0)^{cd}	0.0 (0.9)^{c}	20.0 (26.6)^{a}	13.3 (21.3)^{c}	73.3 (58.9)^{c}	0.0 (0.9)^{b}
	0.5	17.4 (24.6)^{d}	0.0 (0.9)^{c}	32.7 (34.8)^{cd}	12.4 (20.5)^{d}	6.7 (15.0)^{b}	0.0 (0.9)^{c}	11.3 (19.6)^{c}	86.6 (68.5)^{d}	6.7 (15.0)^{a}
	0.1	13.1 (21.1)^{e}	0.0 (0.9)^{c}	29.5 (32.9)^{d}	10.7 (18.9)^{d}	6.7 (15.0)^{b}	0.0 (0.9)^{c}	10.5 (18.9)^{c}	93.3 (75.0)^{e}	0.0 (0.9)^{b}
	Control	-	0.0 (0.9)^{c}	-	-	0.0 (0.9)^{d}	0.0 (0.9)^{c}	-	100.0 (90.0)^{f}	0.0 (0.9)^{b}
	5.0	42.2 (40.5)^{b}	13.3 (21.2)^{b}	50.5 (45.3)^{b}	29.3 (32.7)^{a}	6.7 (9.5)^{b}	20.0 (26.6)^{a}	31.8 (34.3)^{ab}	60.0 (50.8)^{b}	0.0 (0.9)^{b}
	3.0	33.1 (35.1)^{c}	13.3 (21.2)^{b}	39.5 (38.9)^{c}	25.6 (30.3)^{a}	0.0 (0.9)^{c}	6.7 (15.0)^{c}	29.4 (32.8)^{bc}	73.3 (58.9)^{c}	6.7 (15.0)^{a}
Methanol	1.0	28.7 (32.4)^{d}	6.7 (12.6)^{c}	35.2 (36.3)^{c}	15.9 (23.2)^{b}	6.7 (12.6)^{b}	0.0 (0.9)^{d}	26.4 (30.9)^{c}	79.9 (63.4)^{d}	6.7 (15.0)^{a}
	0.5	17.9 (25.0)^{e}	0.0 (0.9)^{d}	23.4 (28.9)^{d}	13.3 (21.2)^{bc}	13.3 (21.2)^{a}	6.7 (15.0)^{c}	23.2 (28.8)^{d}	80.0 (63.4)^{d}	0.0 (0.9)^{b}
	0.1	12.2 (20.4)^{f}	0.0 (0.9)^{d}	12.1 (20.2)^{e}	11.0 (19.3)^{c}	0.0 (0.9)^{c}	0.0 (0.9)^{d}	20.1 (26.6)^{e}	100.0 (90.0)^{e}	0.0 (0.9)^{b}
	Control	-	0.0 (0.9)^{d}	-	-	0.0 (0.9)^{c}	0.0 (0.9)^{d}	-	100.0 (90.0)^{e}	0.0 (0.9)^{b}
	5.0	34.3 (35.8)^{b}	0.0 (0.9)^{c}	34.3 (35.8)^{b}	26.8 (31.1)^{a}	20.0 (26.1)^{a}	20.0 (26.6)^{a}	21.0 (27.2)^{b}	60.0 (50.8)^{b}	0.0 (0.9)^{b}
	3.0	29.1 (32.6)^{c}	20.0 (26.6)^{b}	30.6 (33.5)^{b}	11.7 (19.8)^{b}	0.0 (0.9)^{c}	13.3 (21.4)^{b}	15.2 (22.9)^{c}	66.7 (54.8)^{c}	0.0 (0.9)^{b}
Aqueous	1.0	21.3 (27.5)^{d}	0.0 (0.9)^{c}	22.4 (28.1)^{c}	9.6 (17.8)^{bc}	6.7 (12.6)^{b}	20.0 (26.6)^{a}	11.5 (19.8)^{d}	73.3 (58.9)^{d}	0.0 (0.9)^{b}
	0.5	15.8 (23.4)^{e}	0.0 (0.9)^{c}	18.1 (25.1)^{cd}	8.5 (16.7)^{bc}	0.0 (0.9)^{c}	6.7 (15.0)^{c}	10.4 (18.8)^{d}	93.3 (75.0)^{e}	0.0 (0.9)^{b}
	0.1	9.6 (18.0)^{f}	0.0 (0.9)^{c}	13.7 (21.7)^{d}	7.4 (15.8)^{c}	0.0 (0.9)^{c}	0.0 (0.9)^{d}	9.4 (17.8)^{d}	100.0 (90.0)^{f}	0.0 (0.9)^{b}
	Control	-	0.0 (0.9)^{c}	-	-	0.0 (0.9)^{c}	0.0 (0.9)^{d}	-	100.0 (90.0)^{f}	0.0 (0.9)^{b}
	1.0	42.0 (40.4)^{a}	13.3 (21.4)^{b}	28.3 (32.0)^{a}	18.7 (25.6)^{a}	20.0 (26.6)^{a}	6.7 (15.0)^{a}	21.5 (27.6)^{a}	40.0 (39.2)^{a}	20.0 (26.6)^{a}
	0.7	33.9 (35.6)^{b}	26.7 (31.0)^{a}	25.2 (30.1)^{a}	13.2 (21.0)^{b}	6.7 (12.6)^{b}	0.0 (0.9)^{b}	16.3 (23.7)^{b}	53.3 (46.9)^{b}	13.3 (21.4)^{b}
	0.5	27.8 (31.8)^{c}	20.0 (26.1)^{ab}	20.1 (26.6)^{b}	10.1 (18.4)^{bc}	13.3 (21.2)^{a}	6.7 (15.0)^{a}	13.4 (21.3)^{c}	60.0 (50.8)^{b}	0.0 (0.9)^{d}
Saponin	0.3	20.3 (26.8)^{d}	6.7 (12.6)^{c}	14.5 (22.4)^{c}	9.3 (17.7)^{c}	6.7 (12.6)^{b}	6.7 (15.0)^{a}	13.0 (21.1)^{c}	79.9 (63.4)^{c}	0.0 (0.9)^{d}
	0.1	14.6 (22.5)^{e}	0.0 (0.9)^{d}	9.9 (18.2)^{d}	8.6 (17.0)^{c}	20.0 (26.1)^{a}	0.0 (0.9)^{b}	12.4 (20.6)^{c}	80.0 (63.4)^{c}	0.0 (0.9)^{d}
	0.05	11.8 (20.1)^{f}	6.7 (12.6)^{c}	9.2 (17.5)^{d}	5.8 (13.8)^{d}	13.3 (21.2)^{a}	0.0 (0.9)^{b}	11.3 (19.7)^{c}	93.3 (75.0)^{d}	6.7 (15.0)^{c}
	Control	-	0.0 (0.9)^{d}	-	-	0.0 (0.9)^{c}	0.0 (0.9)^{b}	-	93.3 (75.0)^{d}	6.7 (15.0)^{c}

Values in the parentheses are angular transformed values. Means within a column followed by the same letter are not significantly different [$P < 0.05$; Duncan's (1995) multiple range test]

RESULTS AND DISCUSSION

Among the various extracts of *M. indica* evaluated against *H. armigera* by leaf dip method, the maximum percent antifeedence was caused by methanol extract that ranged from 42.2 to 12.2 followed by aqueous extract (34.3 to 9.6) in the dose ranging from 5.0 to 0.1 percent (Table1). None of the extracts caused the larval mortality more than 20.00 percent .The mean larval weight reduction (3DAT) at 5% concentration was maximum of 50.5 percent by methanol extract followed by hexane (46.6) and aqueous extract (34.3), The larval weight reduction was more on 3 DAT than 7 DAT in all the concentration tested. Highest larval pupal intermediates were recorded in aqueous extract of *M. indica* where as the pupal mortality was at par by all the extracts at 5.0 percent concentration.

The percent antifeedancy observed with saponin as a pure compound from *M. indica*, applied in various concentrations differed significantly from each other (Table 1). While maximum of 42.3 percent antifeedancy was recorded at 1.0% minimum of 11.8 percent was observed at 0.05% level.

The pure compound, saponin at 0.7% recorded 26.7 percent laval mortality. The higher concentration 1.0% caused only 13.3 percent larval mortality. The larval weight reduction ranged ranged between 28.3 – 9.2 and 18.7 – 5.8 at 3 and 7 DAT respectively. The larval- pupal intermediates and pupal mortality did not show any dose dependent activity. The maximum of 21.5% pupal weight reduction was observed at 1.0%, which differed significantly from others.

Data recorded on the adult emergence revealed that most of the treatments recorded lower adult emergence as compared to the control. Minimum normal adults (40.0%) emerged from 1% pure compound saponin, followed by hexane (59.9%), aqueous and methanol extract (60.0%), which were statistically at par. The maximum deformed adults 20.0% were recorded in the pure compound at the highest concentration evaluated that is 1.0%.

The values of AI_{50} and GI_{50} and I_{50} of various extracts of *M. indica* seed against *H. armigera* are given in Table 2. It is evident from the table that amongst the various extract methanol extract was found to be more effective in causing antifeedency with AI_{50} value of 7.14%.

Table 2. Relative effect of various extracts of *M.indica* on antifeedancy, growth and development of *H.armigera*

Extracts	AI 50 %	GI 50 % on 3 DAT	I 50 %
Hexane	42.3 at 10%	9.32	9.83
Methanol	7.14	5.40	8.0
Aqueous	44.3 at 10 %	45.2% at 10 %	53.3 at 10 %
Saponin	42.0 at 1.0 %	28.3 at 1.0 %	0.75

AI_{50} = Antifeedant index; $GI_{50=}$ Growth inhibition index; $I_{50=}$ Inhibition of normal adults

The hexane, aqueous extract and saponin at the highest concentration showed less than 50.0% antifeedency. Methanol extract was more effective in reducing the larval weight gain against *H. armigera*, GI_{50} value being 5.40% followed by hexane extract (9.32%). The aqueous extract and the saponin at the highest concentration produced less than 50.0% larval weight reduction. When the larvae of *H. armigera* were fed with various extracts of *M. indica*, saponin inhibited normal adult emergence by 50.0% at 0.75% followed by methanol (9.80%) and hexane extract (9.83%).

Application of mahua oil significantly reduced the damage caused by rice leaf folder *Cnaphalocrosis medinalis* (Rajasekaran *et al*., 1987) green leaf hopper *Nephotettix virescens* (Mariappan *et al.,* 1988) on rice crop, aphid population on lime tree (Jothi *et al*., 1990) and *H.armigera* in pigeon pea Akhauri and. Yadhav 1999. Even though, mahua oil was found effective in many occasions there is no comparative study of all the extract. Thus it is concluded that saponin and methanol extract of *M. indica* was more effective to cause percent antifeedancy, larval growth inhibition and inhibition of normal adult development against *H. armigera* than hexane and aqueous extracts.

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(Accepted : 20 May, 2006)