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Growth regulatory and toxic effects of non-edible oil seed extracts and purified extracts against *Helicoverpa armigera* (Hubner)

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Crude and purified extracts of non edible seed.of *Pongamia glabra* (Karanja) were tested for their toxicity growth and development against the 2nd instar larvae of the American bollworm, *Helicoverpa armigera* (Hubner) a serious pest of cotton. Larvae reared on synthetic diet at concentrations of 1 and 0.5% of *P. glabra* exhibited 100% mortality, whereas at sublethal concentrations of 0.05% and 0.01% of *P.glabra*, the survival of the larvae, were significantly reduced thereby affecting the normal adult emergence. The reduction in larval weights between the treatments was also highly significant.

Key Words: *Helicoverpa armigera, , Pongamia glabra*, Toxicity,Botanical pest control agents.

Introduction

Indiscriminate use of synthetic insecticides has resulted in problems like pest resurgence, environmental pollution, destruction and toxicity to non-target organisms including, human beings. This resulted in greater attention on bioactivity of phytochemicals as potential insecticides particularly against phytophagous insects. *Helicoverpa armigera,* a polyphagous pest, plays an important role in reducing the crop yields. Although the pest has been reported to develop resistance to several insecticides (Armes *et al.,* 1992; Chandel and Chander 1995). Some of these insecticides are still used for its control due to non-availability of alternative insect control agent. These problems have led to the search for more sustainable, cost-effective and eco- friendly alternatives for pod borer management. In our continuing research programme on the development of insect control agents from plants, we have screened crude and purified extracts obtained from the non edible seeds of *Pongamia glabra,* some of which

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were found promising against this important pest. Non edible seed oils from other plants are also known for their biological activity. (Banerji and Misra 1985).

Materials and methods

Extraction of seeds: Seeds of *P. glabra* were collected from western ghat, Maharashtra, India, shade dried powdered and processed as follows: powdered seeds of 1kg were extracted with acetone followed by methanol (2Lx3 each) at room temperature. From the combined extracts, the solvent was evaporated separately and the extracts obtained were dried to yield an acetone extract (KA 97 g, 9.7% based on the weight of seeds) and methanol extract (KB 56.9 g, 5.69 %).

Purification of extracts: Extract KA 50g was partitioned between aqueous methanol (85 %), 200 ml and pet-ether, 400 ml. The petroleun-ether solubles and methanol solubles were separated from which solvent was evaporated to yield petroleun-ether solubles KA_1 40g (7.76%) and methanol soluble KA_2 10g (1.04%). Extract KB 36.9g, was treated with acetone 50 ml x3 at room temperature and the acetone solubles were filtered off , from which solvent was evaporated to yield acetone soluble, KB_1 20.0g (3.08%) and acetone insoluble KB_2 16.95 (2.52 %).

Formulations of extracts and purified extracts: KA_1 1 g and Tween 20 400 mg were mixed mechanically at room temperature for 30 minutes to yield an emulsifiable concentrate (EC). To this EC 50 ml of aqueous ethyl alcohol was added to obtain a stable emulsion. Similarly KA_2 and $KB_1$1g each ,were formulated as mentioned above to yield a stable emulsion. KB_2 1g was dissolved in 40 ml aqueous ethyl alcohol to obtain a clear solution.

Isolation of Karanjin: KB_1 5g was chromatographed on silica gel (200g 60-120 mesh) using acetone: petroleum ether gradient. The fraction containing karanjin was further purified by preparative TLC and crystallized to yield 200mg of a pale yellow crystalline solid which was identified as karanjin (KR), by comparing its TLC pattern, melting point and NMR with those of reference sample of karanjin.

Formulation of Karanjin: Karanjin 100 mg, was emulsified as mentioned above by using 2.5 ml of dimethyl sulfoxide 0.1 ml of isobutanol and 100mg of Tween 20. This was diluted to 10 ml with water to obtain emulsion of Karanjin (KR). Blank control was prepared simultaneously.

Biological Assay: Solutions and emulsions were diluted further with water to obtain the required concentration of test samples. Four concentrations of all the test samples were prepared as 1, 0.5, 0.1 and 0.05 %. The synthetic diet was prepared (Nagarkatti and Prakash 1974) and aliquots of 10 g of this

diet was impregnated at 30^0C with various concentrations of the samples and mixed thoroughly. The control diet was prepared using blank formulations. For toxicity studies, the solidified diet was cut into pieces and offered to pre-weighed *H.armigera* larvae (2^{nd} instar). Twentyfive replicates were used for each treatment. The experiment was repeated three times. Untreated controls were run simultaneously. The vials were kept at 27°C and 60-70% RH, 14:10: (L:D). Mortality count after24 hrs was noted. Lethal concentration of 50 % larval mortality (LC_{50}), for each sample was calculated. For studies on survival, early 2^{nd} instar larvae weighing 10-15 mg were used. At sub-lethal concentrations, the larvae were allowed to feed *ad libitum* on the treated diet throughout their larval period. The weight of larvae on 3^{rd}, 5^{th} and 7^{th} day were noted and mean weight gain of each treated groups were calculated. Comparative growth, ratio of weight in treated diet relative to control was also calculated. Daily observations on larval mortality (LM), pupal mortality(P M) malformed pupae (MP), deformed adults (DA) and normal adult emergence (NA) were recorded. The adults were morphologically identical with the counterparts emerging in control regarded as normal. The effective concentration for 50% normal adult emergence inhibition (EI_{50}) was calculated for each sample. Mortality at various stages of morphogenesis and abnormal adults were considered in totality to arrive at EI_{50} values. Percent inhibition of emergence of adult E.I was calculated by the following formula (Mulla and Darwazeh 1975).

$$\% EI = 100 - T/C \times 100.$$

Where: T is the emergence of adults in treatment and C is the emergence of adults in control.

Data was statistically analysed by using Student 't' Test. EI_{50} and LC_{50} were calculated by subjecting the data to probit analysis(Finney 1971).

Larvae of *H. armigera* when exposed to various concentrations of formulations exhibited toxicity, such as larval mortality (LM) after 24 hrs or just before pupation, pupal mortality and deformed pupae and adult mortality. In some case the anterior portion of the larvae unsclerotised. Few larvae moulted into deformed/ and malformed (MP) pupae which were much smaller in size than the control pupae. The deformed adults (DA) were characterized by the presence of either undersized wings or crippled wings or exuvae still attached with the body.

Results and discussion

Out of various seed extracts of *P.glabra,* except KA_1 all extracts exhibited 100% mortality at 1 and 0.5 %. At sublethal concentration of 0.1 and 0.05 %,all extracts of *P.glabra* exhibited mortalities at various stages of development. KA_1 was found to exhibit 10-12 % larval-mortality, malformed pupae and deformed adults (Table. 1). The larval period was prolonged by10 days. In case of KR and KA_2, 10-20 % deformed larvae, malformed pupae and deformed adults were observed. The normal pupae were only 10 % as compared to 96 % pupation in control. The larval period was prolonged in 10-17 days. In the case of KB_1 and KB_2, the larval period was prolonged in 10-12 days and there were only 0-10 % deformed larvae. The pupation was only 15-20 % and they were malformed. About 10- 30 % deformed adults were emerged.

Table 1. Effect of extracts and purified extracts of *Pongamia. glabra* seeds on inhibition of adult emergence of *Helicoverpa armigera.*

Extracts	Concentration (%)	E.I (%)
KA_1	1	100
	0.5	100
	0.1	61.76
	0.05	48.64
KA_2	1	100
	0.5	100
	0.1	90.0
	0.05	69.13
KB_1	1	100
	0.5	100
	0.1	79.58
	0.05	69.94
KB_2	1	100
	0.5	100
	0.1	69.68
	0.05	58.90
KR	1	100
	0.5	100
	0.1	90.04
	0.05	84.79
Control	5	2.5

%EI- Percentage Emergence Inhibition

KA_1: Pet ether soluble of Acetone extract; KA_2 : Methanol soluble of Acetone extract; KB_1: Acetone soluble of Methanol extract and KB_2 : Acetone insoluble of Methanol extract of Karanja seeds; KR: Karanjin

Adult emergence was reduced at higher and sublethal concentrations in case of all the extracts. There was 100 % inhibition of adult emergence. Even at sublethal concentration of 0.1 and 0.05 %, they were found to inhibit 50-90 % adult emergence: Among the extracts tested, KR was found to be most active. It exhibited lowest LC_{50} (0.080 %) and EI_{50} (0.0117 %). Activity wise the fractions were graded as $KR>KA_2>KB_2>KB_1>KA_1$ (Table. 2).

Table 2. EI_{50} and LC_{50} values of various seed extracts of *P. glabra* for 2nd instar of *Helicoverpa armigera.*

Fraction	Regression equation	EI_{50}	Fiducial limits 95% (Upper-lower)	Regression equation	LC_{50}	Fiducial Limits 95% (Upper-lower)
KR	-1.4126x + 6.510	0.0117	0.0370-0.0096	2.5627x + 0.0927	0.080	0.1216-0.04310
KA_1	-0.9371x + 6.587	0.0494	0.6344-0.2761	2.3627x + 1.6655	0.257	0.3921-0.1056
KA_2	-1.5714x + 6.809	0.01417	0.0413-0.0120	1.6973x + 1.6963	0.088	0.1162-0.0078
KB_1	-1.2493x + 6.553	0.0175	0.19611-0.0725	2.3729x + 2.069	0.171	0.2661-0.04170
KB_2	-1.1179x + 6.658	0.0304	0.0524-0.0178	2.5699x + 1.5209	0.225	0.3136-0.1770

KA_1: Pet ether soluble of Acetone extract; KA_2 : Methanol soluble of Acetone extract; KB_1: Acetone soluble of Methanol extract and KB_2 : Acetone insoluble of Methanol extract of Karanja seeds; KR: Karanjin

Result revealed that extracts of *P.glabra* (KR, KA_2 and KB_1) adversely affected growth of the larvae which was found to be dose dependent. At 1 and 0.5 % concentration the larvae could not grow beyond one day. At lower concentration of 0.1 %, there was a considerable reduction of larval weights than the control larvae (382.25 mg) after 7th day of feeding. The comparative growth was highly significant ($P<0.005$). In case of KB_2 at the highest dose of 1% the larval period was only for 1-3days. The comparative growth was significant ($P<0.005$) at 0.5 %. In KA_1 growth was significantly affected only at 1 and 0.5 % concentration ($P<0.005$). The larval weights were five times less than the weights of larvae in control (Table3).

Table 3. Effect of various extracts of seeds of *P.glabra* on growth of 2nd instar larvae of *Helicoverpa armigera*.

Extracts	Con (%)	Mean larval weight (mg)				(%) Comparative growth
		0 day	3rd day	5th day	7th day	
KA_1	1	12.08 ± 0.96	14.69 ± 0.32	*	*	0.704^{++}
	0.5	13.39 ± 0.28	16.22 ± 1.92	55.74 ± 2.18	94.99 ± 2.90	22.07^{++}
	0.1	12.49 ± 0.29	98.31 ± 2.12	121.31 ± 2.01	184.93 ± 2.64	46.52^{+}
	0.05	14.02 ± 0.20	141.20 ± 3.08	253.98 ± 4.36	298.98 ± 4.55	76.88
KA_2	1	12.78±0.81	*	*	*	0
	0.5	14.23±0.38	*	*	*	0
	0.1	13.34±0.89	53.76±1.99	64.39±2.23	71.88±2.10	15.79^{++}
	0.05	14.10±0.95	68.38± 2.52	164.85±4.73	242.50± 3.72	61.65
KB1	1	14.31±1.19	*	*	*	0
	0.5	13.70± 0.89	*	*	*	0
	0.1	12.56± 0.99	94.06± 2.89	90.29± 2.10	121.05± 3.2	15.79^{++}
	0.05	14.10± 1.12	183.59± 4.52	121.74± 3.80	280.66± 3.5	61.65
KB_2	1	12.32±1.10	*	*	*	0
	0.5	13.14±1.08	14.31±1.10	*	*	0.31^{++}
	0.1	12.22±1.32	93.96±2.6	100.52±3.1	152.65±2.1	37.89^{+}
	0.05	14.04±1.10	130.64±3.9	205.59±4.8	289.91±3.8	74.43
KR	1	0	*	*	*	0
	0.5	13.50±1.80	*	*	*	0
	0.1	12.46±1.19	44.93±2.65	48.90±1.70	53.0±2.93	10.95^{++}
	0.05	13.50±1.15	63.24±2.65	160.42±2.79	221.94±3.82	56.22
Control	5	12.58±0.82	163.50±2.	225.08±2.8	383.20±3.8	100

Values significantly different from control analyzed by Student t test
++ P<0.005 +P<0.001 * Larval mortality
KA_1: Pet ether soluble of Acetone extract; KA_2: Methanol soluble of Acetone extract; KB_1:Acetone soluble of Methanol extract and KB_2: Acetone insoluble of Methanol extract of Karanja seeds.

The findings reveal that at higher concentration of 1%, extract inflicted 100% larval mortality. Similar toxic effects was reported for oil of Calamus (Risha 1990 and Su 1991a). The toxicity attributed to *P.glabra* was due to Karanjin molecules which contains hydroxyflavone called Pongamol (Perry 1980) or 4,5 dihydroxy-1-methyl-2 piperidine carboxylic acid (Southon and Buckingham 1988). At sublethal concentrations mostly all the extracts exhibited various abnormalities effectively. Such type of effects were reported against *Dysdercus similes* and *C. cephalonica* (Kumar and Thakur 1989; Chouhan *et. al*., 1987) with other non edible oi1s. The retarded growth of the larvae has direct influence on the weights of the body. Similar effects of weight loss in case of *Callosobruchus chinensis* reported with *P.glabra* oil (Ketkar 1986).

Various abnormalities at larval, pupal and adult stage and toxic effects suggest the interference of these extracts with growth and development processes. Since morphogenetic hormones regulate these processes , it can be

inferred that these extracts interfere with morphogenetic hormones of the insects. These extracts caused toxicity at various stages of development , thereby inhibiting overall population of adults. Such type of effect where emergence of adults are inhibited as reported on *C.cephalonica* (Chander and Ahmed 1986), and *T. castaneum* (Joseph et. al 1994). It is evident from the foregoing discussions that formulations of *P.glabra* showed great promise in controlling the pest *H. armigera* owing ease of extracting, and plentiful availability. On the basis of insect toxicity, the findings of present study clearly revealed the effective control of *H. armigera* by these formulations of *P.glabra* as a potential control measure for the management of the American bollworm, (*H. Armigera)* if it aimed at younger instars. The efficacy can further be increased by using additives and synergists.

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