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Research Article

Biopesticidal and Larvicidal Effects of *Chukrasia tabularis* A Juss Extracts on Third Instar Larva of *Helicoverpa armigera*

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ABSTRACT

Chukrasia tabularis A.Juss plant belongs to Meliaceae family. Limonoids are such compounds abundantly present in *C.tabularis* and being tetranotriterpenoid in nature .The present paper deals with biopesticidal effect of *C.tabularis* seed oil and seed various extract on *Helicoverpa armigera*. Highest mean percent reduction over control was highest with methanol (43.07 to 85.94%), followed by ethanol (41.15 to 68.85%) and petroleum ether (39.18 to 66.14%) at all the three concentrations tested. Among the different concentrations tested, mean percent reduction in population over control increased as the concentration of the extracts increased with maximum reduction at 500 ppm. For seed oil methanol at 500 ppm (85.9%) followed by ethanol (68.85%) and petroleum ether (66.14%) recorded highest percent reduction over control, aqueous extract and benzene at 125 ppm found least effective, however effectiveness increased at 500 ppm.

Keywords: *C.tabularis* , *Helicoverpa armigera* , seed oil, Biopesticidal activity, Botanical pesticides.

INTRODUCTION

Biopesticides generally affect only the target pest and closely related organisms, in contrast to broad spectrum, conventional pesticides. They are effective in very small quantities and often decompose quickly, thereby resulting in lower exposures and largely avoiding the pollution problems caused by conventional pesticides. Plant secondary compounds have been the subject of thorough investigation for the past 30 years in an effort to discover new sources of botanical insecticides and antifeedants. Among the plant families studied, the Meliaceae, Rutaceae, Asteraceae, Labiateae, Piperaceae and Annonaceae are perhaps the most promising[1-3]. The Meliaceae and Rutacae have received much attention at least partly owing to the presence of triterpenoids called limonoids[4]. Azadirachtin, a limonoid from seeds of the neem tree (*Azadirachta indica*, Meliaceae), possesses strong antifeedant and growth inhibitory effects against various insect pests[5]. *Melia volkensii* contains limonoids related to azadiractin. Crude *M. volkensii* fruit extract is toxic to a broad range of insects including dipterans, lepidopterans, and coleopterans extracted from bark of the chinaberry tree, *Melia azedarach* (syn. *Melia toosendan*) is another example of a limonoid used commercially for its insecticidal properties[6]. Some of the other promising families are Lamiaceae, Annonaceae, Apiaceae and Asteraceae. *Oreganum vulgare* (Lamiaceae,) usually known as oregano, has documented antifungal[7], antiviral, and antibacterial[8] properties. Furanocoumarins (characteristic of the Apiaceae) (e.g. xanthotoxin), are known to be widely toxic to generalist insect herbivores[9] and as potent antifeedants for a number of insects[10,11]. Digitoxin and cymarin are cardiac glycosides or cardenolides. Cardenolides are known as oviposition deterrents[12,13]. Phenol, is a major constituent of garden thyme, *Thymus vulgaris* (Lamiaceae) and *Origanum vulgare* (Lamiaceae) and trans-anethole, a phenylpropanoid from the anise plant, *Pimpinella anisum*, are toxic to *Spodoptera litura*[14]. The toxicity of trans-anethole has also been demonstrated against number of species, including various beetles, weevils, and mosquitoes[15-17].

Chukrasia tabularis Adr. Juss.

It is known as Indian Mahogany wood or Red cedar wood. Locally it is known as '*Kondavepa*' or '*Godlavepa*' (in Telugu), The genus is represented by one to two species from India eastwards to Malesia and south China; one in India[18].

Description

Densely foliaceous with extensive crown; - Height upto 30mtrs; - Girth to 5mtrs; Bark-dark grey or rusty brown; Flowers-cream, April to June (September), fragrant; Fruit-massive, woody, brown in colour, persistent, dehiscing by next February, capsule globose and septifiragal; Seeds-many, flattened, broadly winged below, exalbuminous, cotyledons orbicular and Mostly evergreen, but becomes leafless in cold weather.

Distribution World

Nepal, Sri Lanka, Pakistan, Bangladesh, Bhutan, India, Burma, (Hajra et.,al 197)

India

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West Bengal, Sikkim, Arunachal Pradesh, Assam, Tripura, Meghalaya, Maharashtra, Andhra Pradesh, Karnataka, Tamil Nadu, Kerala and the Andaman and Nicobar Islands[19].

Andhra Pradesh

Talakona and Tirumaia ofChittoor district and Cuddapah district. Phenolic compounds act as natural pesticides, providing plants with resistance to pathogens, parasites, and predators[20,21]. Limonoids are such compounds and being tetranotriterpenoid in nature, they are included in the category of phenols. These compounds occur in abundance in the plants belonging to family meliaceae. Abdelgaleil and Nakatani, isolated several types of compounds (limonoids) as insect antifeedent from the members of meliaceae family[22-25]. In a leading step to their research, further isolated 6 new phragmalin limonoids (tabulalin and tabulalides from the root bark of *C. tabularis* using droplet countercurrent chromatography (DCCC) and reversed phase HPLC.

MATERIALS AND METHODS

Extraction of oil from the seed

The seed that was used for the extraction of oil was 10 kg. Oil was extracted using a table expellor at Oil Technological Research Institute (OTRI) JNTU, Anantapur, Andhra Pradesh, India. The oil that was extracted in a table expellor.

Preparation of solvent extracts of seed

The preparation of solvent extracts of seed oil as follows below.

Preparation of solvent extracts of Chukrasia seed

100 g. of seed was weighed and crushed to powder in a blender. This powder was taken in a sufficient conical flask (with a small mouth to prevent evaporation of the solvent) and 300 ml. of the solvent was added, (1:3 ratio) mixed well, closed and kept for 24 hours. Then, the solvent was removed and again, 300 ml. fresh solvent was added and the same process was repeated for three times in 72 hours. During the process, the active ingredients of seed get into the solvent. Finally, the total pooled solvent of three washes was collected in a clean container. This aggregate methanol was subjected to evaporation in a rotary evaporator (model, Laborota 4000 by Heidolf instruments) to get a crude extract which contains the soluble components of *Chukrasia* seed in the respective solvent. This process was done for the preparation of crude solvent extracts of methanol, ethanol, hexane, petroleum ether, ethyl acetate, benzene and water (aqueous extract). All these respective solvent extracts of seed were stored in an amber colored sample bottles in a refrezirator at 4^0C[26].

Preparation of stock solutions of extracts of seed

Stock solutions were prepared by weighing 250 mg. of each solvent extract (Methanol, Ethanol, Petroleum ether, and Ethyl acetate, Benzene, Hexane and Water) of seed, meal and oil and were made up to 10 ml. in a 10 ml. standard flask with 10% DMSO solution (Dimithylsulfoxide). These are 25000 ppm (10%) concentrated stock solutions from which further dilutions were prepared with water.

Rearing of Insect Pests

Rearing of Helicoverpa armigera

The glassware and other equipment used were cleaned and sterilized. The glassware and plastic troughs were cleaned with clean, sterile water. Subsequently, they were rinsed with 0.1 percent mercuric chloride solution to avoid from microbial contamination. Finally, they were cleaned with cotton swab dipped in 4.0 percent formalin solution. Eggs of *H. armigera* (Hubner) (Lepidoptera- Noctuiidae) were collected on a moist filter paper in a petridish of 4" diameter from the Dept. of entomology of Acharya N.G.Ranga Agricultural University, ANGRAU, Anantapur. These eggs were placed in the above sterilized containers closed with muslin cloth for aeration. The eggs hatched in three days. Tender, young leaves of muskmelon were provided as feed for the neonate larva. For every 24 hours, fresh leaves were placed as feed. The larva entered third instar stage on 8th day. Major damages by *Helicoverpa* is done from this stage onwards 8-13 mm long and the larvae in this third instar stage were used for the biopesticidal investigation because the first and second instar stages of larvae are delicate and are easily amenable for the control with insecticides[27].

Experimental Design and Statistical Analysis

Experiments were conducted both in field and lab conditions. Field study was conducted with 8 (solvent extracts including control) × 3 (concentrations of each extract) factorial experiment arranged in a Randomized Block Design (RBD). Laboratory experiments with all the eight solvent extracts including control and three concentrations were arranged in a Completely Randomized Design (CRD). Number of replications used was three. Data was analyzed using WindowStat version 8 (Indostat services, Hyderabad, India) statistical software with split plot analysis and two factorial RBD. Wherever necessary ARC SIN transformation was used for obtaining normalized data. Means for field studies were compared with least significance difference (L.S.D) test at P=0.05 level of probability for significance or non-significance of main effects (solvent extract type) and sub effects (concentrations) and their interactions. Means for laboratory enzyme studies were compared with Tukey's Honestly Significance Difference (Tukey's H.S.D) test at P=0.01 level of probability for significance of main effects and sub effects and their interactions[28,29].

RESULTS AND DISCUSSION

Different solvent seed extracts of *Chukrasia tabularis* tested for mean percent reduction in population over control of *H. armigera* under lab conditions resulted in significant effect of solvent type and concentration of extracts. Highest mean percent reduction over control was highest with methanol (43.07 to 85.94%), followed by ethanol (41.15 to 68.85%) and petroleum ether (39.18 to 66.14%) at all the three concentrations tested. Among the different concentrations tested, mean percent reduction in population over control increased as the concentration of the extracts increased with maximum reduction at 500 ppm. Although statistically, there is no significant interaction between solvent type and concentrations, methanol at 500 ppm (85.9%) followed by ethanol

Table 1: Effect of different solvent extracts of *Chukrasia tabularis* (seed) on third instar larva of *Helicoverpa armigera* under lab conditions.

Extracts	Concentration (ppm)		
	Percent reduction in population over control		
	125	250	500
Methanol	55.6 (48.84)	76.3 (62.40)	100.0 (85.94)
Hexane	47.3 (44.04)	73.0 (56.83)	92.6 (74.90)
Ethanol	54.3 (46.92)	75.0 (60.26)	96.6 (81.15)
Petroleum ether	51.0 (45.01)	74.6 (57.85)	95.3 (76.35)
Ethyl acetate	49.3 (44.04)	71.0 (56.83)	92.6 (73.40)
Benzene	46.6 (43.08)	69.3 (55.76)	92.0 (71.95)
Aqueous Extract	43.6 (40.17)	68.0 (53.76)	89.6 (68.66)
	SEM	CD (P=0.05)	Significance
Solvent Extracts	1.40	6.08	**
Concentrations	0.69	2.90	**
Interaction			
Solvent Extracts × Same level of Concentrations	1.87	7.19	NS
Concentrations × Same level of Solvent Extracts	2.08	8.42	NS

Values in parenthesis are ARC SIN transformed values *and NS indicates significant (P=0.05) and non significant respectively.

Table 2: Effect of different solvent extracts of *C.tabularis* oil on third instar larva of *Helicoverpa armigera* under lab conditions.

Extracts	Concentration (ppm)		
	Percent reduction in population over control		
	125	250	500
Methanol	45.6(43.07)	67.3(55.81)	100.0(85.94)
Hexane	39.3(38.22)	62.0(50.78)	82.5(65.00)
Ethanol	42.3(41.15)	66.0(53.84)	87.6(68.85)
Petroleum ether	41.0(39.18)	64.6(51.75)	86.3(66.14)
Ethyl acetate	39.3(38.22)	60.0(50.78)	81.6(64.69)
Benzene	37.6(37.25)	59.3(49.80)	81.0(63.54)
Aqueous Extract	33.6(34.18)	57.0(47.87)	77.6(61.14)
	SEM	CD (P=0.05)	Significance
Solvent Extracts	1.16	5.04	**
Concentrations	0.53	2.08	**
Interaction			
Solvent Extracts × Same level of Concentrations	1.41	5.52	**
Concentrations × Same level of Solvent Extracts	1.64	6.75	**

Values in parenthesis are ARC SIN transformed values *indicates significant at P=0.01

(68.85%) and petroleum ether (66.14%) recorded highest percent reduction over control, aqueous extract and benzene at 125 ppm found least effective, however effectiveness increased at 500 ppm. Advances in chemical and biotechnological techniques enable us to understand the mode of action of these botanical pesticides thereby enhance the speed and ease with which man can discover and develop secondary compounds of plants origin pesticides. These advances, combined with increasing need and environmental pressure are greatly increasing the interest in the use of plant products as pesticides. Thus, *Chukrasia tabularis* can be used as a botanical insecticide which can be routed as one of the viable alternatives to synthetic chemical insecticides for integrated pest management as these natural compounds pose little threat to the environment or to human health. The search for plant-derived chemicals that have potential use as crop protectants (insecticides, antifeedants, growth inhibitors) often begins with the screening of plant extracts. Initially, the test insects are fed with the extracts and effects on insect behavior and development are monitored. Once a promising extract has been discovered, the next step is to find out how it is affecting the insect; i.e. what is its mode of action? This kind of information is needed to ensure safety to non-target organisms (humans, beneficial insects)[30]. Results of the present study could possibly pave way towards exploitation of plant products from *C. tabularis* as a novel chemotherapeutant in plant protection.

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