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PEST MANAGEMENT

Biological Effects of *Caesalpinia crista* Seed Extracts on *Helicoverpa armigera* (Lepidoptera: Noctuidae) and Its Predator, *Coccinella septumpunctata* (Coleoptera: Coccinellidae)

Emmanuel Nathala* and Swaran Dhingra

Division of Entomology, Indian Agricultural Research Institute, New Delhi-110012

Abstract *Caesalpinia crista* seed extracts were investigated in the laboratory against *Helicoverpa armigera* (Hubner). The extracts exhibited powerful antifeedant and growth disruption activity. The toxic symptoms of the poisoned larvae included reduction in weight gain and mortality of larvae and pupae, larval-pupal intermediates and malformed adults. Maximum antifeedance is caused by methanol extract (AI_{50}=0.0186%), followed by hexane extract (0.0212%), ethyl acetate extract (0.0416%), butanol extract (0.0767%) and aqueous extract (0.2199%). The larval mortality ranged from 10.00 to 70.00% in different extracts. The 50% larval growth inhibition recorded at 3DAT was statistically at par by methanol and hexane extract. The percent I_{50} values for inhibiting normal adult emergence were 0.0287, 0.0325, 0.0485, 0.0977 and 0.0547% for methanol, hexane, ethyl acetate, aqueous and butanol extract. The biosafety evaluation of these extracts carried out against the predator, *Coccinella septumpunctata* showed no mortality of the adults till nine days after treatment. Though the observation taken at 10 DAT, showed slight mortality of adults by methanol extract at both 5.0 and 1.0% concentration.

Keywords *Helicoverpa armigera*, *Caesalpinia crista*, Antifeedance, IGR, *Coccinella septumpunctata*

Introduction

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. They contain terpenoids (Schmutterer, 1984) that are phagodeterrent (Pradhan *et al.* 1962), growth inhibitors (Rembold *et al.*1980) and oviposition suppressant (Jacobson *et al.* 1978).

Caesalpinia crista Linn., known as karanjwa in India, is a perrinial shrub branches finely-downy, leaflets membranous, elliptic-oblong, obtuse; petiolules very short; hooked spines. Flowers in dense (usually spicate) long-peduncled terminal and superaxillary recemes dense at the top, lax downward. Pods shortly stalked, oblong, densely armed on the faces with prickles. Distributed throughout India, generally

*Corresponding author.
E-mail: emmanuelnathala@rediffmail.com
Tel: +91-11-25437224; Fax: 0091-11-25849747

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tropically and in Asia, Australasia, Indian Ocean Islands, and Pacific Ocean Islands. Gonzalez and Lagunes (1986) revealed that in field and laboratory trials *Caesalpinia pulcherrima* extracts have reduced the *S. frugiperda* damage on maize plants. Osman *et al.* (1995) reported that *C. pulcherrima* extracts were highly deleterious to *Corcyra cephalonica* in inhibiting adult emergence, enhancing development time from larva to adult and reducing fresh weights of adult females. Kardinan *et al.* (1997) observed that *C. sappan* leaves, seeds, and flower powder at 2.5% (w/w) each caused mortality of *Callosobruchus analis*.

Earlier, Akhtar *et al.* (1985) and Javed *et al.* (1994) reported the antihelminthic property of *C. crista* seed extracts against ascarid infection in buffalo calves and poultry. From the dichloromethane extract of seeds kernel of *C. crista*, four cassane type furano diterpenes have been reported. These have been characterized as caesalpinins and its analogues. Some other compounds identified from the seeds include, nor caesalpinin-E, caesalmin-C and its desacetyl and desacetoxy derivatives, caesaldekarin and its acetoxy derivatives (Kalauni *et al.* 2004).

Materials and Methods

Seed collection and Processing

Seeds were procured from the forests of Madhya Pradesh (India). These were manually broken to separate the kernel from the seeds coat and sun dried for 2-3 days. Dried kernels were ground to powder and were used for extraction with solvents of different polarity.

Extraction

The *C. crista* seed kernel (CSK) powder (500 g) was extracted with hexane (2×3 lit) using mechanical stirrer for half an hour and the blend was kept overnight for 24 hrs. The supernatant was then filtered and subjected to vacuum distillation at 45℃ to obtain hexane extract concentrate (132.77 g). The defatted powder residue was then extracted with methanol (2×3 lit). Blend was thoroughly stirred for half an hour and kept over night for 24 hrs. The supernatant was filtered and subjected to vacuum distillation at 45℃ to yield methanol extracts concentrate (56.16 g). The methanol extract concentrate (50 g) was then partitioned with ethyl acetate: water (3:1). The organic layer was concentrated under vacuom (45℃) to obtain viscous material (2.07 g). The resultant cake obtained after hexane and methanol extraction was suspended in distilled water (2 lit) and stirred for half an hour. The mixture was kept overnight for 24 hrs, filtered, concentrated and partitioned with butanol: water (1:1). The butanol extract was filtered and distillated under vacuom at 50℃ to obtain viscous material (2.65 g). In another experiment, fresh CSK powder (500 g) was extracted with distilled water (3×1×2 lit) as above. The supernatant after 24 hr was filtered, concentrated under vacuom at 50℃ to obtain viscous material (23.19 g).

Insects

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

Biological testing

Feeding assay

Antifeedant effect of various extracts of *C. crista* seed kernel was assessed on 3rd instar larvae (30-40 mg) of *H. armigera* by artificial diet incorporation method. The extracts were weighed and added into the diet after it has cooled down to 50℃. The ingredients were stirred thoroughly by using a mini electric blender. The treated diet discs (20 mm dia. and 10 mm thick) were transferred individually to clean petri plates (8 cm×1.5 cm). The normal diet was used as control. One larva starved for one hour was introduced in each petri plate. Each treatment and control was replicated ten times. Observations on the amount of diet consumed were recorded at 72 hrs after treatment. The percent antifeedance was calculated using the following Abbot's modified formula:

$$\text{Percent diet protection} = \frac{\text{Total diet given (mg)} - \text{diet consumed (mg)}}{\text{Total diet given (mg)}} \times 100$$

$$\text{Percent Antifeedance} = \frac{\text{Percent diet protection in treatment} - \text{Percent diet protection in control}}{100 - \text{Percent diet protection in control}} \times 100$$

Effect on development of larvae, pupae and adult.

Larvae were allowed to feed on the test diets till the

completion of their larval period. The development of the treated larvae was monitored up to adult emergence. Data were recorded on percent 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 antifeedance index (AI_{50}), growth inhibition index (GI_{50}), median lethal concentration (LC_{50}), and inhibition of normal adult emergence (I_{50}). The percent growth reduction of larvae and pupae over control was computed as follows:

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

Effect on adults of *C. septumpunctata*

The adults of *C. septumpunctata* collected from field were preconditioned in the laboratory for 3-4 h. Ten randomly selected beetles were transferred to clean glass specimen tubes (10 cm × 4 cm) containing a mustard twig infested with aphids (*Lypaphis erisyimii*) which was previously sprayed with various concentrations of each of the extracts of *C. crista* seed. Each treatment including control was replicated three times. Mortality counts were taken every day till 10 days after treatment.

Results and Discussion

Antifeedant activity

The percent antifeedance obtained at different concentrations of various extracts against 3rd instar larvae of *H. armigera* is shown in fig 1. It is seen that all the extracts of *C. crista* seeds, significantly deterred feeding by larvae. The percentage of antifeedance ranged from 29.26 to 80.97% in hexane, 22.01 to 90.95% in methanol, 19.77 to 75.49% in ethyl acetate, 20.40 to 58.01% in butanol and 11.86 to 45.79% in aqueous extract. Methanol extract was found to be the most effective (AI_{50}=0.0186%) followed by hexane extract (0.0212%), ethyl acetate (0.0416%), butanol (0.0767%) and aqueous extract (0.0876%) (Table 1).

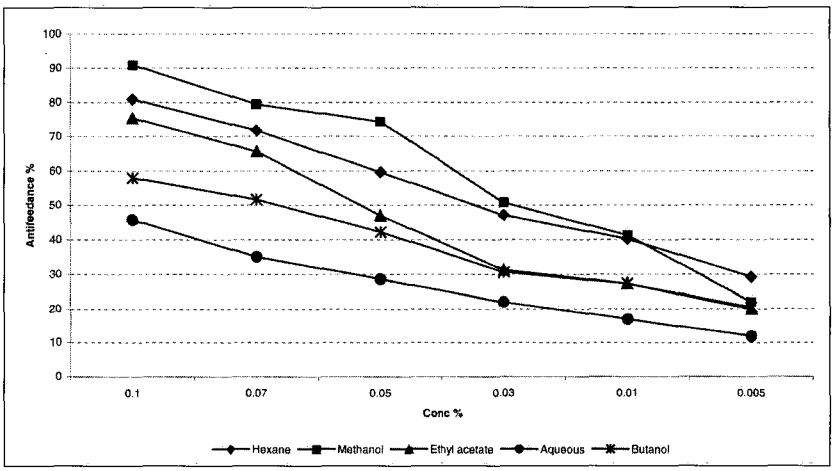

Fig 1. Effect of *C. crista* seed extracts on the feeding by 3rd instar larvae of *H. armigera.*

Table 1. Comparative efficacy of different extracts of *C. crista* seeds on feeding, survival, growth and development of *H. armigera*

Extracts	AI_{50} (%)	LC_{50} (%)	GI_{50} (%)		I_{50} (%)
			3 DAT	7 DAT	
Hexane	0.0212	0.0555	0.0206	0.0570	0.0325
Methanol	0.0186	0.0236	0.0151	0.0241	0.0287
Ethyl acetate	0.0416	0.0762	0.0405	0.0633	0.0485
Aqueous	0.2199	0.0891	0.0729	0.0727	0.0547
Butanol	0.0767	0.1247	0.0867	0.1074	0.0977

AI_{50}=The concentration required for causing 50% antifeedance, GI_{50}=The concentration required for causing 50% larval weight gain reduction over the control, LC_{50}=The concentration required for causing 50% larval mortality, I_{50}=The concentration required for causing the 50% normal adult emergence inhibition

Toxicity to insect larvae

C. crista seed extracts adversely affected survival of larvae in a dose dependent manner. The percent cumulative larval mortality ranged from 13.33 to 63.34% in hexane, 23.33 to 70.00% in methanol, and 16.67 to 56.67% in ethyl acetate, 16.67 to 53.33% in aqueous and 10.00 to 50.00% in butanol extract (Fig 2). On the basis of LC_{50} value, the decreasing order of toxicity of various extracts against the larvae was methanol (0.0236%) > hexane (0.0555%) > ethyl acetate (0.0762%) > aqueous (0.0891%) > butanol extract (0.1247%) (Table 1). Relative toxicity of various extracts of *C. crista* seeds calculated by taking aqueous extract as unity, revealed that hexane, methanol and ethyl acetate extract was 1.60, 3.77 and 1.67 times more effective than that of aqueous extract, while the butanol extract was 0.71 times as effective as aqueous extract. In all cases however, mortality was delayed and temporarily staggered. Most treated larvae died in their old cuticle. In some cases the old cuticle was partially shed, with remnants remained attached to the new cuticle. Rupture of imperfectly formed new cuticle was sometimes observed.

Disruption of larval, pupal and adult growth.

Continuous feeding on the treated diet discs with various extracts of *C. crista* seeds induced various deformities in larvae, pupae and adults of *H. armigera*. A concentration dependent percent larval weight reduction was observed at 3 and 7DAT (fig 3). At 3DAT, the 50% growth reduction in the treated larvae was observed with methanol extract (GI_{50}=0.0151%) followed by hexane (0.0206%) and ethyl acetate extracts (0.0405%) (Table 1). The percent larval weight reduction at 7DAT was significantly less than at 3 DAT. The larvae that fed on the diet discs treated with various extracts of *C. crista* seeds resulted in abnormal pupation. They were unable to ecdyse successfully or to produce a normal pupal cuticle, resulting in larval pupal intermediates (LPI). LPI and pupal mortality form all the extracts ranged from 3.33 to 6.67%. The mean percent pupal weight reduction are shown in fig 4. At the highest concentration the pupal weight reduction was maximum with methanol extract (40.36%) followed by hexane extract (37.58%), ethyl acetate extract (35.75%), aqueous extract (33.10%) and butanol extract (32.52%).

There was a prolongation in the larval and pupal duration due to the treatment by various extracts. The larvae required 5.33, 5.89, 3.83, 1.91, 2.00, and 3.00, 5.39, 3.74, 2.74, 1.67 additional days over control to reach the pupal and adult stage by hexane, methanol, ethyl acetate, aqueous and butanol extract, respectively.

The percent normal adults emerged in each of the extracts are shown in fig 5. All the extracts significantly reduced the normal adult emergence compared to control. The percentage of normal adults emerged ranged from 20.00 to 83.34% in hexane, 20.00 to 76.67% in methanol, 30.00 to 83.33% in ethyl acetate, 40.00 to 90.00% in butanol, 36.66 to 80.00% in aqueous extract. The 50 percent normal adult emergence inhibition (I_{50}) values of hexane, methanol, ethyl acetate and butanol extract were 0.0287, 0.0325, 0.0485, and 0.0977%, respectively (Table 1). Interestingly aqueous extract inhibited the same at 0.0547%, which has a potential to play a role in pest management in sustainable agriculture production.

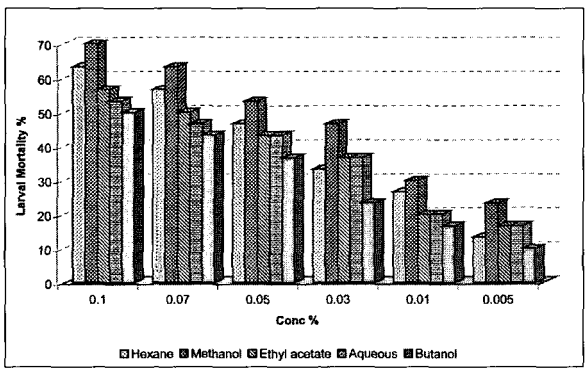

Fig 2. Effect of *C. crista* seed extracts on *H. armigera* larval survivability.

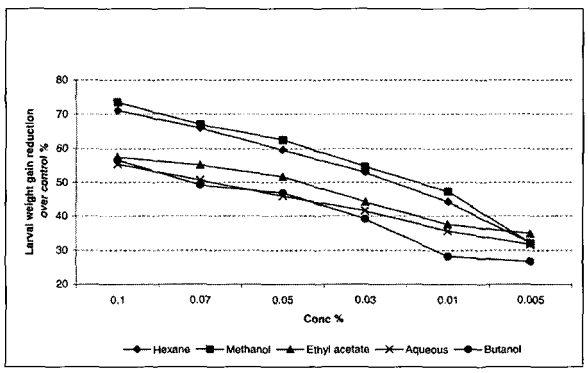

Fig 3. Effect of *C. crista* seed extracts on the larval weight gain reduction (3DAT) of *H. armigera* larvae.

Effect on adults of *C. septumpunctata*

The extracts of seeds of *C. crista* did not cause any mortality till nine days after treatment. The observation taken at 10 DAT, showed that none of the extracts of *C. crista* seeds caused mortality of adults at either 5.0 and 1.0% concentration, except for methanol extract, which caused 3.33% mortality at the same concentrations.

Results from these studies clearly confirm the potential insect-control properties of the extracts of *C. crista*. The reduction in growth rate, formation of larva-pupal intermediates, larval and pupal mortality and the adult malformation, following the treatment with extracts of *C. crista* may be consequence of number of physiological events. Antifeedant studies have shown that at the lower dose (0.005%) the extracts of *C. crista* does not significantly reduce food intake in the larvae that fed on the treated diet discs, implying that that reduction in growth rate cannot be attributed to the antifeedant action of the compound. Consideration of the weights of pupae obtained in this study support this contention, as larvae that fed on the treated diet discs with low doses of the material produced pupae of comparable weight to controls, suggesting that they did not experience any severe feeding inhibition. At higher dose (0.1%) extracts of *C. crista* acts as an antifeedant and considerably smaller pupae are formed. Mortality in insects was delayed and death in such insects could more reasonably be attributed to extreme metabolic and physiological aberrations than to any direct action of the extracts of *C. crista*. The production of larval-pupal intermediates would also imply a possible interference by the extracts of *C. crista* with the juvenile hormone titres and activity, as these usually determine the outcome of interstage moults in most insects (Wigglesworth, 1972). Further hormonal systems that may be affected by the extracts of *C. crista* here might be those relating to the eclosion hormone (Seiber and Rembold, 1983). The mortality of insects during eclosion observed in our work could thus be attributed to such interference. The antifeedant and toxic effects of extracts of *C. crista* observed in the present study can be attributed to the one or more of the many bioactive compounds contained in the seeds of *C. crista*. The compounds that may interact with the feeding, growth and development of the insect are caesalpinins and its analogues, norcaesalpinin-E, caesalmin-C and its desacetyl and desacetoxy derivatives, caesaldekarin and its acetoxy derivatives (Kalauni *et al.* 2004). The present study shows that the crude extract of *C. crista* seeds has a strong antifeedant and toxic activity against *H. armigera*. But whether a single compound or several compounds in the plant seeds are responsible for the obtained results remains to be discovered. However, because *H. armigera* is a generalist pest, the present study may acquire a special emphasis, as generalist herbivores are considered less sensitive to deterrents than specialists (Bernays *et al.*, 2000), implying that *C. crista* seed extracts could be even more potent against specialist caterpillars.The predator, *C. septumpunctata* adults were safe when fed on the aphids treated with extracts various extracts of seeds of *C. crista*, which can be utilized under field conditions without the loss of natural enemy population. It is generally accepted that antifeedants can be used effectively as a part of an integrated pest control program. A field assessment of the detterency and toxicity of the extracts of *C. crista* seeds should provide helpful information for the development of entomo-toxic materials from the plant.

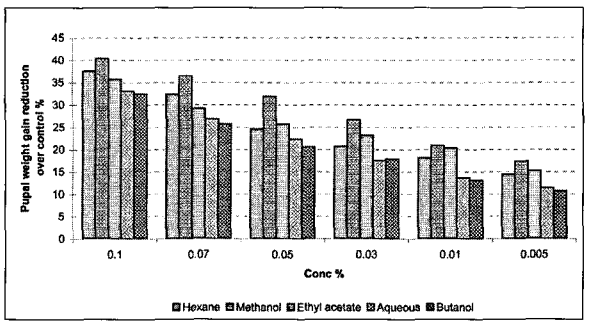

Fig 4. Effect of *C. crista* seed extracts on the pupal weight gain reduction over control of *H. armigera*

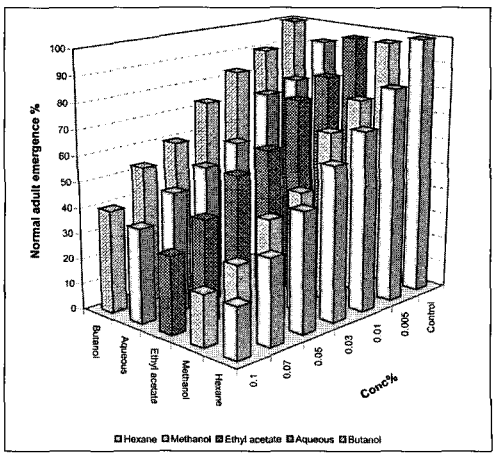

Fig 5. Effect of *C. crista* seed extracts on the normal adult emergence of *H. armigera*

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