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## Larvicidal activity of different solvent extracts from the seeds of *Abrus precatorius* (L.) against pod borer, *Helicoverpa armigera* (Hubner)

S Ramesh Babu, Prahlad Kumar Meena and Ramgopal Dudwal

### Abstract

The present study was conducted on solvent extracts of seeds from *Abrus precatorius* against *Helicoverpa armigera*. Significant weight reduction in the surviving larvae when they were fed with different concentrations of solvent extracts of seeds from *Abrus precatorius* when compared to untreated control. Despite continuous feeding with a normal diet, larvae fed on *A. precatorius* methanol 10% treatment failed to recover to the normal weight. After 9 days of the experiment, their maximum weights ranged from 0.021g+0.020 to 0.257 g+0.100 as compared to the control larvae (0.364 g+0.051). In all the treatments the larvicidal activity was directly proportional to the concentration of the extract. Among the solvent extracts of *A. precatorius* seeds, at higher concentrations of 10% methanol extracts induced a significant decrease in larval weight and survival at 7<sup>th</sup> and 9<sup>th</sup> day. At 10% of methanol extracts, larvae could not gain sufficient weight and showed 100% larval mortality and gave the highest insecticidal activity when compared to other solvent extracts like ethyl acetate and hexane in which larvae could recover after fed with control diet within two to three days. These results indicated that the methanol extracts from *Abrus precatorius* affects the survival of *H. armigera*.

**Keywords:** *Helicoverpa armigera*, *Abrus precatorius*, larval weight, methanol

### 1. Introduction

*Helicoverpa armigera* (Hübner) (Lepidoptera; Noctuidae) is distributed from Europe, Africa, Asia and Australasia to the New World [1]. In India, this pest is polyphagous in nature and present throughout the year and larval stage can survive and feed on 181 cultivated and wild plant species from about 45 families [2]. Some of them are important agricultural crops like cotton, sorghum, groundnut, pigeonpea, chickpeas, tomato etc. Losses up to Rs.10, 000 million have been reported solely due to this pest in these crops [3, 4].

Insecticide resistance and resurgence in different crops led to the indiscriminate use of insecticides against *H. armigera*. This pest shows resistance to all the major insecticide classes and it has become increasingly difficult to control its population in India [3]. This has necessitated a search for more eco-friendly approaches to managing this pest. Plant based compounds have been isolated and extracted for their biological activities against pests and diseases and to find out a new mode of action and to develop active agents based on natural plant products, efforts are being made to isolate, screen, and develop phytochemicals possessing pesticidal activity. These categories of pesticides are now known as biopesticides. Plant based pesticides are highly toxic to many insect species and more than 2000 plant species are known to possess some insecticidal activity [5].

In recent years, there has been a growing interest in the exploiting plant extracts for their insecticidal activities for different pests. The secondary metabolites play an important role in insecticidal, hormonal and antifeedant activities of many plant extracts and their bioactive compounds of many several insects have been demonstrated [6].

Crab's eye, *Abrus precatorius* (L.) is a vine native to India and also known as jequirty bean, Rosary pea, and love bean. *A. precatorius* belongs to the family Fabaceae. The whole plant is poisonous, and seeds are more used for various purposes. The various toxic constituents isolated from various parts of the plant are reported as N-methyltryptophan, flycyrhizin (lypolytic enzyme that is the active principle of liquorice), abrin (toxalbumin, also known as phytotoxin), abrine as alkaloid (amino acid), abralin (glucoside), and abric acid [7].

*Abrus precatorius* has wide-range of numerous bioactive and the insecticidal properties which have been investigated for over a hundred years [8, 9].

The aim of this study was to use laboratory assays to evaluate solvent based (hexane, ethyl acetate and methanol) seed extracts of *A. precatorius* for toxicity towards *H. armigera* by assessing the growth inhibitory effect and mortality.

## 2. Materials and Methods

**2.1. Plant material:** The seeds of *Abrus precatorius* were collected from nearby villages of Banswara district Southern Rajasthan India.

**2.2. Extraction of seed crude extracts:** The seeds were dried in the laboratory at room temperature for one week. The dried seed material was pulverized into fine powder using a mixer-grinder. Five-hundred-gram seed powdered material was taken and soaked in n-hexane in a bottle for 72 h with occasional shaking for solvent crude extraction. The same procedure was repeated with other solvents viz., ethyl acetate and methanol sequentially and the extracts obtained with each solvent were filtered through vacuum pump and the respective solvents were evaporated (at 40°C) with the help of Rotary vacuum evaporator (Heidolph, Germany) [10]. The semi-solid and powdered substances were obtained and stored in refrigerator at 4°C for further use.

**2.3. Insect Culture of *Helicoverpa armigera*:** The initial culture (adults and larvae) was collected from the chickpea fields of Agricultural Research Station, borwat farm and maintained in the insect rearing laboratory under controlled conditions. The set of pupae were also procured from the National Bureau of Agriculturally Important Insects, Bengaluru (National accession no: NBAII-MP-NOC-01). A temperature regime of 25±1°C and 60-70% RH were maintained in the laboratory. This culture room was backed up by a BOD incubator also. The larvae were maintained in the artificial diet prepared from the following ingredients, chickpea seed flour (110 g); yeast powder (20 g); casein (10 g); methyl-p-hydroxy benzoate (2 g); sorbic acid (0.5 g); formaldehyde (1 ml); ascorbic acid (2.6 g); cholesterol (0.115 g); streptomycin sulphate (0.1 g); multivitamin mixture (1 g); vitamin E (0.6 g); bacto-agar (12 g); distilled water (720 ml) [11].

## 2.4. Growth inhibitory activity of solvent crude extracts of *A. precatorius*

The stock solutions of respective solvent extracts were diluted further with water to obtain required concentration of 5.0 and 10.0% of test samples and this was compared with Neem formulation, NSKE 10%. The crude extracts of different solvent extracts of seeds of *A. precatorius* were incorporated as per above mentioned two concentrations in the artificial diet of *H. armigera* [12]. Control diet was prepared without the above said solvent extracts. Starved larvae of *H. armigera*

(n=24, 35-44 mg) were released into rearing trays containing control diet or solvent extracts containing diet. Larval weights were recorded at the same time on different alternate days, mean weight gain of each groups and percentage comparative growth in relation to control were calculated. Fresh diet was added as and when the larvae required or every alternate day. Data on pupal weight, malformed pupae and adults were recorded.

**2.5. Statistical Analysis:** Data were statistically analyzed by using Student's t test. Significant differences between treatments were determined by Tukey's tests ( $P \leq 0.05$ ) (Source: Minitab 18 Statistical Software).

## 3. Results and Discussion

Early 3<sup>rd</sup> instar larvae of *H. armigera* showed larval mortality, reduction in growth and development as well as larval-pupal intermediates when exposed to various concentrations of solvent extracts of seeds of *A. precatorius*. The effects of hexane, ethyl acetate and methanol solvent extracts on growth and development of *H. armigera* were evaluated initially at different concentration at 5 to 10% and are shown in Table 1. Significant differences among the treated larvae were observed after exposure to different solvent extracts in the diet. Larval mortality, reduction in growth and development, % pupation and malformed adults were exhibited when early 3<sup>rd</sup> instar larvae of *H. armigera* exposed to different concentrations of solvent extracts of *A. precatorius*.

The average weights of larvae fed on solvent extracts of *A. precatorius* seeds after 10-11 days of incubation are shown in Table 1. Significant differences among fractions were observed. There was significant weight reduction in the surviving larvae when they were fed with different concentrations of solvent extracts of *A. precatorius* when compared to untreated control. Despite continuous feeding with normal diet, larvae fed on *A. precatorius* methanol 10% treatment failed to recover to the normal weight. After 9 days of experiment, their maximum weights ranged from 0.021g+0.020 to 0.257 g+0.100 as compared to the control larvae (0.364 g+0.051). In all the treatments the larvicidal activity was directly proportional to the concentration of the extract.

Among the solvent extracts of *A. precatorius* seeds, at higher concentrations of 10% methanol extracts induced a significant decrease in larval weight and survival at 7<sup>th</sup> and 9<sup>th</sup> day. At 10% of methanol extracts, larvae could not gain sufficient weight and showed 100% larval mortality and gave the highest insecticidal activity when compared to other solvent extracts like ethyl acetate and hexane in which larvae could recover after fed with control diet within two to three days. At a concentration of 10%, NSKE (Neem Seed Kernel Extract) showed high larval.

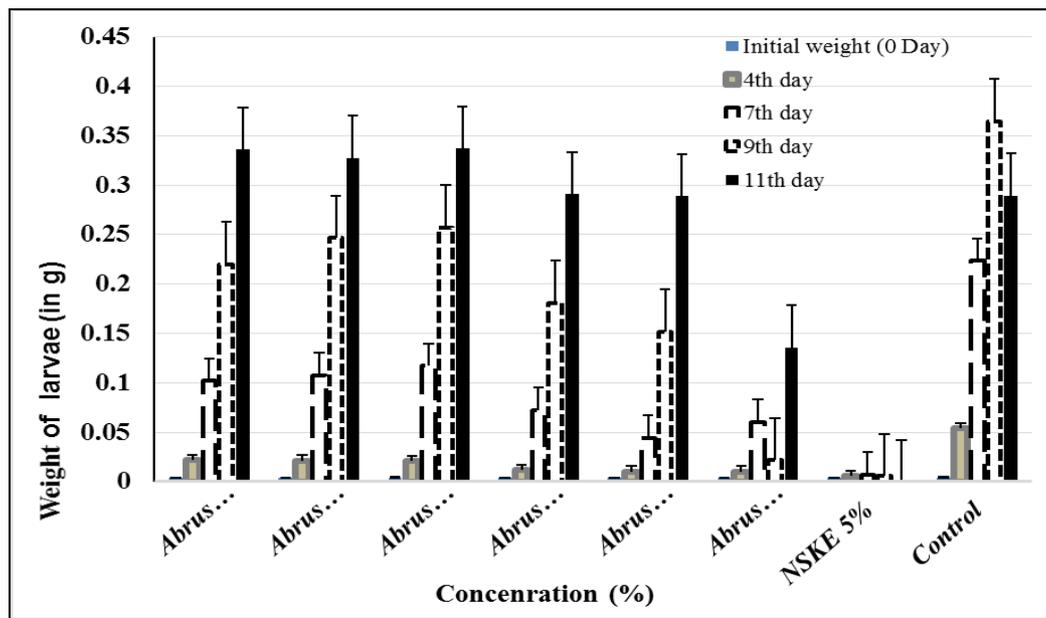
**Table 1:** Effect of different solvent extracts of seeds from *Abrus precatorius* against pod borer, *Helicoverpa armigera*

Solvent extract	Initial Weight (0 Day) (g±SD)	4 <sup>th</sup> day (g±SD)	7 <sup>th</sup> Day (g±SD)	9 <sup>th</sup> Day (g±SD)	11 <sup>th</sup> day (g±SD)	Comparative growth (%)
<i>Abrus precatorius</i> Hexane 5%	0.0036+0.0110 <sup>a</sup>	0.0218+0.0110 <sup>b</sup>	0.101+0.041 <sup>bcd</sup>	0.219+0.084 <sup>bc</sup>	0.336+0.039 <sup>a</sup>	92.30
<i>Abrus precatorius</i> Hexane 10%	0.0038+0.0010 <sup>a</sup>	0.0210+0.0101 <sup>b</sup>	0.107+0.042 <sup>bc</sup>	0.246+0.091 <sup>b</sup>	0.327+0.072 <sup>a</sup>	89.87
<i>Abrus precatorius</i> Ethyl acetate 5%	0.0043+0.0011 <sup>a</sup>	0.0205+0.0095 <sup>b</sup>	0.117+0.049 <sup>b</sup>	0.257+0.100 <sup>b</sup>	0.336+0.036 <sup>a</sup>	92.28
<i>Abrus precatorius</i> Ethyl acetate 10%	0.0035+0.0007 <sup>a</sup>	0.0118+0.0056 <sup>bc</sup>	0.072+0.040 <sup>cde</sup>	0.180+0.089 <sup>bc</sup>	0.290+0.080 <sup>ab</sup>	79.73
<i>Abrus precatorius</i> Methanol 5%	0.0039+0.0009 <sup>a</sup>	0.0103+0.0044 <sup>bc</sup>	0.044+0.024 <sup>ef</sup>	0.151+0.066 <sup>c</sup>	0.288+0.060 <sup>ab</sup>	79.10
<i>Abrus precatorius</i> Methanol 10%	0.0035+0.0010 <sup>a</sup>	0.0104+0.0039 <sup>bc</sup>	0.060+0.029 <sup>de</sup>	0.021+0.020 <sup>d</sup>	*	*
NSKE 5%	0.0038+0.0008 <sup>a</sup>	0.0056+0.0015 <sup>c</sup>	0.007+0.001 <sup>f</sup>	0.005+0.001 <sup>d</sup>	*	*
Control	0.0043+0.0005 <sup>a</sup>	0.0536+0.0252 <sup>a</sup>	0.223+0.050 <sup>a</sup>	0.364+0.051 <sup>a</sup>	control larvae at pre-pupal stage	100

Means followed by same alphabet do not differ significantly by Tukey's test ( $P = 0.05\%$ )

growth inhibition when compared other concentrations. Maximum larvicidal activity observed in the methanol extract of *Caesalpinia bonducella* extracts against *H.armigera* as observed by [13]. The significant larvicidal activity of

*Spodoptera litura* was recorded from the highest concentration of methanol extract at 500 ppm as reported by [14].



**Fig 1:** Effect of solvent extracts from the seeds of *Abrus precatorius* on the larval weight (mean $\pm$  SE) of *Helicoverpa armigera*

Methanol extract of *Abrus precatorius* at 500 ppm showed maximum larvicidal activity of *Spodoptera litura* when compared to other solvent extracts. LC50 value of hexane, diethyl ether, dichloromethane, ethyl acetate and methanol extract of *Abrus precatorius* were 255.91, 266.21, 265.98, 251.84 and 225.76 ppm respectively against fourth Instar larvae of *S. litura* [15]. The methanol extract was responsible for the strong lethal activity observed against selected pest species as reported by Mathivanan *et al.* 2015 [14].

Per cent comparative growth was noted in different solvent extract treatments of *A. precatorius* seeds which vary from 79 to 92% when compared to control (Table 1). Whereas, Pawar *et al.* 2004 [16] reported low percentage comparative growth in *H. armigera* due to different concentrations of seed extracts of *Madhuca latifolia* and *Calophyllum inophyllum* as compared to control. Similarly, methanol stem extracts of *T. neriifolia* shown 46-83% reduction in growth of early IV instars of *H. armigera* than the hexane extract as reported by Mishra *et al.* 2015 [17].

Pupation and pupal duration was increased upto 3-4 days in the solvent extracts of *A. precatorius* seeds when compared to control in which larvae were pupated in 9-10 days (Table 1). It is due to the toxic compound on the juvenile hormone which ultimately arrest the development which makes the larvae to take more time for pupation [18]. Solvent seed extracts of *A. precatorius* showed antifeedant activity for the larvae of *S. litura* and also *S. litura* shown extended larval duration and more time for pupation when fed on solvent seed extracts of *A. precatorius* when compared to control as observed by Arivoli and Tennyson, 2013 [12].

At a concentration of 5 and 1.0%, all seed solvent extracts exhibited 10-20 % larval-pupalintermediates and deformed pupae and 10-15% deformed adults were emerged (Fig. 2). But the pupation percentage and pupal weight was not significant among the treatments and equal to that of untreated control (Table 2). Similarly, the earlier reports on *H. armigera* with different plant species shown that maximum insecticidal activity recorded in ethyl acetate extract of

*Solanum pseudocapsicum* on *H.armigera* [18]. *Strychnos nuxvomica* hexane and chloroform seed extracts showed 31.43% and 22.14% malformed adults, respectively. Ethyl acetate and methanol extracts of *Semicarpus anacardium* seed produced 16.67 and 20% deformed adults, respectively in *H.armigera* [19].



**Table 2:** Effect of various solvent extracts of seeds from *Abrus precatorius* against pod borer, *Helicoverpa armigera*

Solvent extract	Pupal Weight (g $\pm$ SD)	Malformed adults
<i>Abrus precatorius</i> Hexane 5%	0.246 $\pm$ 0.026 <sup>a</sup>	0
<i>Abrus precatorius</i> Hexane 10%	0.247 $\pm$ 0.032 <sup>a</sup>	2
<i>Abrus precatorius</i> Ethyl acetate 5%	0.241 $\pm$ 0.028 <sup>a</sup>	2
<i>Abrus precatorius</i> Ethyl acetate 10%	0.221 $\pm$ 0.036 <sup>a</sup>	3
<i>Abrus precatorius</i> Methanol 5%	0.239 $\pm$ 0.031 <sup>a</sup>	3
<i>Abrus precatorius</i> Methanol 10%	0	-
NSKE 5%	0	-
Control	0.289 $\pm$ 0.034 <sup>c</sup>	0

Means followed by same alphabet do not differ significantly by Tukey's test (P = 0.05%)

Plants are a rich source of secondary metabolites like phenols, alkaloids, terpenoids, flavonoids etc., which play a defensive role against insects. Several authors reported the solvent extracts of different plant species against *H. armigera*. Ethyl acetate extracts of *Solanum pseudocapsicum* exhibited highest insecticidal activity against *H. armigera* as reported by Jeyasankar *et al.* 2012 [18]. Whereas, the present study showed highest larval activity in methanol extracts of *A. precatorius*.

This is possible due to the presence of secondary metabolites in this plant may inhibit the metabolic activities of the larvae and ultimately the larvae failed to feed and inhibit the moulting process and finally hinder the development and mortality occurs <sup>[20]</sup>. The effect of alkaloid abrine from *A. precatorius* seeds on mealy bug, *Maconellicoccus hirsutus* was studied by Anitha *et al.* 1999 <sup>[21]</sup>. They observed that reduction in free sugars, bound sugars and protein and also on lipid suggests that abrine could have a drastic effect on the population of *M. hirsutus*.

#### 4. Conclusion

The present study confirms the presence of some secondary metabolites like alkaloid in the solvent extracts of *A. precatorius* seeds which could be used as an alternative to conventional synthetic pesticides. Further investigations are needed to explore and isolate and purification of the secondary metabolites from *A. precatorius* for its effective use in the control of *H.armigera*.

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