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Insecticidal activity of an indigenous plant of Kerala against IV instar larvae of *Helicoverpa armigera* (Hübner)

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Abstract

The cotton bollworm *Helicoverpa armigera* is a serious pest of many economically important crops. Since this pest has become resistant to the conventional synthetic insecticides, newer compounds and formulations are being developed against this insect pest. Many natural compounds isolated from the plants were tested against this pest. In the present study, indigenous plant, widely distributed plants of kerala, namely, *Gliricidia sepium* were screened for their insecticidal activity against fourth instar larvae of the cotton bollworm, *Helicoverpa armigera* (Hübner) under laboratory conditions. The crude extracts of the plants demonstrated a dose dependent increase in bioactivity. Phytochemical analysis of the extracts of the plants, revealed the presence of different phytocompounds each of which is known to have deleterious effect on insect pests. Thus, it may be concluded that the petroleum ether leaf extract of *Gliricidia sepium* possess insecticidal property and can be further investigated for the development of a potent botanical insecticide. According to the results the percentage mortality of *H. armigera* larvae varied among the treatments. From the results it has been noted that the petroleum ether extract was effective against fourth instar larva of *H. armigera* larvae both on contact and by feeding.

Keywords: *Helicoverpa armigera*, *Gliricidia sepium*, plant extract, toxicity, insecticidal activity, Kerala

Introduction

The cotton bollworm *H. armigera* is a highly polyphagous pest and excessive feeder, finally affecting the crop yield. This pest attacks most of the cultivated crops in the Indian subcontinent; also, it causes economic losses up to 70%. Most of the chemicals used to control this pest have different modes of action. Due to the continuous application of the synthetic chemical pesticides, the insects have developed resistance to insecticides (Tossou *et al*., 2019) [2]. Hence, more studies were conducted against this insect pest with biological products which are safer environmentally. Among the pests, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) represents a challenge for agricultural production around the cultivated and non-cultivated plants belonging to more than 47 families, including soybeans, cotton, sorghum, corn, sunflower, peanuts, beans, tomatoes and peppers (Fathipour and Sedaratian., 2013) [3].

In India it is reported to be feeding on 182 plant species across 47 families (Manjunath *et al*.,1985) [1] and causes an annual loss of about Rs. 2,000 crores (Ignacimuthu and Jayaraj, 2003). Plant derived product Ponnem affected the DNA of *H. armigera* which was confirmed by comet assay (Packiam *et al*., 2015) [5].

Fifty percent of all insecticides used in India and China are to control *H. armigera* alone (Lammers and Macleod, 2007) [6] but the continuous and indiscriminate use of insecticides over the years has resulted in the *H. armigera* developing resistance to certain molecules belonging to different classes of insecticides (Mc Caffery, 1998; Chaturvedi, 2007; Yang *et al*., 2013) [7, 8, 9]. Thus, alternatives to the synthetic pesticides are being sought. The search for alternatives to synthetic pesticides has focused the interest on plant derived pest control agents.

Plant-based pesticides or botanicals have many advantages, mainly they have multifarious control mechanisms against pests (Sivagnaname and Kalyanasundaram, 2004) [10] which reduces the possibility of the development of resistance in pests (Liu *et al*., 2000) [11]; secondly, they are target-specific and hence not harmful to humans and beneficial insects; and finally, they are not persistent in nature and hence environment friendly (Shaalan, 2005) [12].

In the present investigation an attempt has been made to screen with different crude extracts of widely distributed plant *Gliricidia sepium* of kerala for their insecticidal activity against fourth instar larvae of H. *armigera*, which has been reported as a major pest of tomato and chickpea in the state (Thakur *et al*., 2006) [13]. Multiple applications of an average dosage of any pest control agent are generally more effective than a single application in overdose. Rotation of insecticides with different modes of action is also recommended to avoid selection for resistant populations (EMBRAPA 2013) [14].

Gliricidia sepium literally known as “Rat poison”.In primary health care sector it is a multipurpose tree with source one of herbal medicine. The larvicidal activity of *Gliricidia sepium* leaves against the fourth in star larvae of Anopheles mosquitoes was demonstrated by the petroleum ether extract (Jasmine *et al*., 2017) [15]. Majority of the plants tested against different larval instars of *H. armige*ra have been reported to demonstrate antifeedant and insecticidal properties (Ramya *et al*., 2008; Wambua *et al*.,2011; Jeyashankar *et al*., 2012; Arivoli and Tennyson, 2013) [16, 17, 19]. Although extensive research has been conducted on the effect of different plant extracts on *H. armigera*, there is limited literature available on the efficacy

of plants like *Gliricidia sepium* which have a wide distribution in the state of kerala and find application in medicinal practices of the local tribal population (Sinha *et al*., 2008; Sohkhlet, 2014) [20, 21]. The current focus is aimed on the leaf of medicinal plant *Gliricidia sepium* of kerala for their insecticidal activity and oral toxicity against fourth instar larvae of H. *armigera* (Hübner).

Materials and Methods

Collection of Plants

The plant selected for the study (*Gliricidia sepium*) were collected from Thrissur district, Kerala, India. The selection of the plants was based on their local abundance, insecticidal properties and uses in traditional practices by the indigenous tribes of the state.

Collection of Test Organism

Cotton boll worm, *Helicoverpa armigera larvae* were collected from the red gram field of Coimbatore district, Tamilnadu, India, during September to November. The insects were reared under laboratory condition in Petri plates by feeding with fresh discs of Ladys finger-controlled condition in laboratory (25+20C and 65% + 5 RH) throughout the study period. The fourth instar larvae were preferred for the experiment as they were voracious feeders.

ExtractionProcedure

Freshly collected leaves of *Gliricidia sepium* were washed with double distilled water thrice and shade-dried at room temperature for 10–15 days. Dried leaves were crushed and ground into fine powder using an electric blender. A voucher of specimen was stored in laboratory for further reference. Powdered plant materials were sequentially extracted with different solvents in a Soxhlet apparatus for 72 hr. The solvents used for extraction included petroleum ether (PE), chloroform (CH), ethyl acetate (EA) and methanol (MT). The respective extracts were filtered with whattman filter paper and dried under reduced pressure using rotary evaporator to yield solid/semisolid residues. The residues were lyophilized to get dry solid mass. The dry weight of the filtered extract was taken. These extracts were dissolved in respective solvents and were then used for phytochemical analysis. The extract was used for further studies.

Phytochemical Analysis

The different extract was subjected to various phytochemical tests to find out the secondary metabolites. The tests were performed for alkaloids, Proteins, Sterols, phenols, Tannis, terpenoids, Sugars, Quinines, Flavanoids and saponins [Harborne (1973), Trease and Evans (1978)] [23]

Insecticidal Activity

Different concentration (0.625mg/L, 1.25mg/L, 2.5mg/L, 5 mg/L) of crude extract were applied dorsally on the fourth instar larvae by an applicator (camel hair brush). Original pods without treatment were feed to the larvae. In each crude extract with different concentrations (0.625mg/L, 1.25mglL, 2.5mg/L, 5mg/L) two hours, pre-starved single fourth instar larvae of *H.armigera* were introduced individually and covered with muslin cloth. Five replicates were maintained for all concentration and the number of dead larvae was recorded after 24 hours up to pupation. Percentage of larval mortality was calculated by Abbott s formula.

$$\text{Abbotts percent corrected mortality} = \frac{\%\text{mortality in treated} - \%\text{mortality in control}}{\%\text{ mortality in control}} \times 100$$

Statistical Analysis

Mortality rate of test insects was expressed as percentage, whereas effectiveness of extract in killing the insect was expressed as LC50 (lethal concentration 50%). Two-way Anova was applied to compare mortality rate between levels of extract concentration and four solvent extracts. The LC50 was determined using Probit analysis.

Results and Discussion

Insecticidal activity of each extracts was assessed by comparing the averages of the leaf consumed in the treated and control leaves. Efficacy of plant extracts was assayed against the fourth instar larvae of *Helicoverpa armigera* for insecticidal activity.

In the present study, Preliminary phytochemical analysis of *G.sepium* leaves extract were done using four solvents (Petroleum ether, Chloroform, Ethyl acetate and Methanol extract). phytochemical analysis were performed to find out the presence secondary metabolites like, Alkalaoid, Saponin,Tannin, Steriod, Flavanoid, Terpenoid, Phlobotannin, Coumanin, cycloglycosides, Total phenol and Quinone In Petroleum ether extract, steroid, flavonoid, terpenoid, coumarin, cycloglycosides, total phenol, quinone and tannin were present. Among these secondary metabolites tannin were found to be strongly present. Whereas, chloroform extract showed the presence of tannin, steroid, terpenoid and cycloglycosides while, acetate extract showed the presence of saponin, steroid, terpenoid, quinone and coumarin and in the Methanol extract tannin, flavonoid, terpenoid, coumarin and total phenol were present and the rest of the compounds were absent (Table-1)

Insecticidal activity of crude extract of leaf of *G.sepium* was studied at different concentration and the result is presented in Table 2. Insecticidal activity of solvent extract were calculated based on larval mortality after 24hrs treatment, high larval mortality normally indicates potential insecticidal activity varied significantly. Data pertaining to the insecticidal activity of *H. armigera* clearly revealed that maximum insecticidal activity 1.33±0.58 was recorded in petroleum ether extract at 5 % concentration and followed by 3.62±0.53 at 2.5% concentration. 4.67±1.45 at 1.25% concentration and least % were observed as 7.33±1.65 at 0.625% concentration against the control 0.00 ±0.00 which was statistically significant at ($p<0.05$) level. Deformities due to the treatment of crude leaves of *G.sepium* at 5% concentration were also noted. Usually, larger doses of plant extract inflict mortality either by inhibiting feeding or reducing digestibility or inhibiting growth. Colour change from green to red and a sedative condition was also observed in the treated larvae. It has been also reported that ethanolic extracts from *G. sepium* showed larvicidal effects against *Tetranychus cinnabarinus* (Sivira *et al*., 2011)] [24]. In addition, *G. sepium* extract revealed to be effective for controlling insect pest *Godasa sidae* of *Mansonia altissima* seedlings (Ayeni *et al.,* 2017) [25]. Bioactive components that have been identified from plant extracts of *G. sepium* which have antiinsecticide effects consist of Alkaloids, Terpenoids, Phenolics, Coumarin, Tannins, Saponins,

Flavonoids, Quinones, Proteins, and Sterols (Jose and Sujatha 2017) [26]. Study reports have showed that, many flavonoids, have shown the toxic effects on various species of organisms. For example biochanin and pinocembrin are known to be toxic to insects especially changes in termite reproduction capacity (Boue and Raina 2003) [27]. Wheareas, pinocembrin was known to show anti-feeding and mortality effects in the larvae of butterfly *Spodoptera frugiperda* (Diaz Napal and Palacios, 2015) [28].

Screening plant extracts for deleterious effects on insect is one of the approaches used in the screening for novel botanical insecticides. Secondary plant compounds act as insecticides by poisoning per se or by production of toxic molecules after ingestion. These compounds also deter or possibly repel an insect from feeding. (Ben jannet H *et al.*,2000). In the present study chloroform extract of leaves of *G.sepium* exhibited a significant Insecticidal activity at a higher concentration.

It is possible that the insecticidal property present in the selected plant compound may arrest the various metabolic activities of the larvae during the development & ultimately the larvae failed to moult and finally death occured. Similar works have already reported on Insecticidal activity of many plants and their compounds against different group of insects (Hashim *et al.,* 2003) [29]. Insect growth regulation properties of plant extracts are very interesting and unique in nature, since insect growth regulators works on juvenile hormone. The enzyme ecdysone plays a major role in shedding of old skin and the phenomone is called ecdysis or moulting.

Table 1: Preliminary phytochemical analysis of *G.sepium*

Secondary metabolites	Solvents			
	Petroleum ether	Chloroform	Ethyl acetate	Methanol
Alkalaoid	–	–	–	–
Saponin	-	–	+	-
Tannin	++	+	-	+
Steroid	+	+	+	-
Flavonoid	+	–	-	+
Terpenoid	+	+	+	+
Phlobotannin	-	–	-	-
Coumarin	+	–	+	+
Cycloglycosides	+	+	-	-
Total phenol	+	–	-	+
Quinone	+	–	+	-

Positive (+) Strongly Positive (++) Negative (−)

Table 2: Mortality of *H. armigera* treated with four different solvent leaf extracts of *Gliricidia sepium* at different concenteration after 24 hrs of treatment.

Concenteration	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Mehanol extract
Control	0.00 ±0.00a	0.00 ±0.00a	0.00 ±0.00a	0.00 ±0.00a
0.625%	1.33±0.58ab	1.00 ±1.05bc	1.00 ±0.00ab	1.33 ±1.13ab
1.25%	3.62±0.53c	3.67 ±1.43bc	2.37 ±0.58b	3.17 ±0.25bc
2.5%	4.67±1.45c	5.30 ±1.00a	3.77±1.35c	5.40 ±1.53b
5%	7.33±1.65bc	6.67±2.71bc	6.33 ±1.53bc	6.00±1.63c

Values in the same row followed by the same superscript are not different at $p < 0.05$ by LSD test

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