Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2018; 6(1): 1744-1746

Received: 18-12-2017
Accepted: 21-01-2018

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Effect of crude extract of *Atalantia monophylla* L. on ovicidal activity against *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) and preliminary phytochemical study

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Abstract
Methanol, acetone and water crude extracts of *Atalantia monophylla* leaf were studied for ovicidal activity against *Helicoverpa armigera*. The sample concentration at 0.1, 0.2, 0.6, 0.8 and 1.0% were used in present experiment. Among the three solvents tested at all concentrations for ovicidal activity, acetone extract 5% was found to be statistically significant(49%) followed by methanol and aqueous leaf extract. Aqueous leaf extract even at 5.0% was noticed least value. The leaf extract tested was exhibited the presence of phytochemicals such as alkaloids, terpenoids, flavonoid, saponins, phenols and anthraquinone in different solvent extracts.

Keywords: *Atalantia monophylla*, *Helicoverpa armigera*, antifeedant, larvicidal

1. Introduction
The green revolution in India was accompanied with the use of chemical pesticides resulted insignificant increasing an agricultural production by controlling the insect pests. The continuous use of chemical pesticides has created chronic effects on living organism and environmental deterioration. However the health consciousness prompts us to restrict the use of many pesticides. Moreover, synthetic Insecticides led to numerous acute and chronic poisoning of applicators, farm workers, consumer, fish, birds and other domestic, wildlife animals etc [1].

Atalantia monophylla L. belongs to the family Rutaceae and is commonly called as wild lime. Various parts of *A. monophylla* have been used in folk medicine, against rheumatoid pain and glandular swelling [2]. The root is believed to be antispasmodic [3] whereas a decoction of the leaves is often applied for itching and other skin complaints. Hexane, chloroform and ethyl acetate extracts of *A. monophylla* showed antifeedant, larvicidal, pupicidal and ovicidal activities against *Helicoverpa. armigera a*nd *Spodoptera litura* [4]. The tribes (Pulayar) of Thadagai hills are using leaves to treat swellings and act as insect repellent. Atalaphyllinine, atalantin, dehydroatalantin, cycloepiatalantin and atalaphylline 3, 5-dimethyl ether have been reported from root bark [5].

Helicoverpa armigera Hubner (Lepidoptera: Noctuidae), cotton bollworm, is one of the most important agricultural crop pests in the world and inhabits diverse ecological habitats, leading to heavy yield losses in a diverse range of crops. It is also widely distributed in India and attacks varied plant species, leading to substantial losses [6]. In the present piece of research, an effect of crude extract of *Atalantia monophylla* L. on ovicidal activity against *Helicoverpa armigera* Hubner (lepidoptera: noctuidae) and preliminary phytochemical study was undertaken.

2. Materials and Methods
2.1. Plant collection and extract preparation
The fresh plant material of *A. monophylla* was collected from Amboli, Kolhapur, Maharashtra, Leaves were collected and shade dried at room temperature and ground in a manual mill. The powder was sequentially extracted with methanol, chloroform, acetone and water. The powder was soaked in the respective solvents for a period of 48 h with intermittent shaken. The extract was filtered through a Buchner funnel with Whatman filter paper number one.

The filtrate was evaporated to dryness under reduced pressure using rotary evaporator. Untreated eggs were used as control.

2.2. Rearing of *H. armigera*

The colony of *H. armigera* was maintained in the laboratory under the controlled conditions of 27 ± 1^0C and $80 \pm 5\%$ relative humidity (RH) with a 12-hour light/dark photo regime. Larvae of *H. armigera* were reared on artificial diet prepared using chickpea flour, casein and yeast as the main components and certain necessary nutrients in traces and agar as the semi-solidifying agents. The neonates of *H. armigera* were reared in groups for about seven days till they develop into third instars. Thereafter, to prevent cannibalism, individual larva was transferred to separate clean and sterilized petri dishes (7 cm diameter) for further rearing. Pupae were sterilized with 0.5% sodium hypochlorite solution, washed with distilled water, and kept inside the plastic cages (15 × 15 cm) for adult emergence. Adults were fed on 10% sucrose solution that was kept in small vials in the cages. After a period of two days, 10 pairs of moths were selected, and each pair was released in a separate oviposition jar (2000 ml) covered with clean muslin cloth on the top for oviposition. The egg-laden cloth was replaced each day with the fresh cloth till the oviposition continued. The eggs collected were then allowed to hatch in a tight-lid box having tiny holes on the lid's surface.

2.3. Ovicidal activity

A muslin cloth piece, containing 20 one-day-old eggs of *H. armigera*, were dipped 0.1, 0.2, 0.6, 0.8 and 1.0% concentrations of methanol, acetone and aqueous extract. Five replicates were evaluated for each concentration. The experiments were conducted at 27±2 °C and 75±5% RH. The number of eggs hatched at different treatments conditions was recorded. The percent ovicidal activity was calculated according to Abbott (1925) [7] after 24, 48 and 96 hours of treatment and the values were recorded.

$$\text{Percent ovicidal activity} = \frac{\%\text{ of eggs hatched in control} - \%\text{ of eggs hatched in treated}}{\%\text{ of eggs hatched in control}} X100$$

2.5. Qualitative test for phytochemical analysis

All solvent extracts of *A. monophylla* were subjected to various chemical tests to identify phytoconstituents using standard methods [8, 9, 10].

2.5.1. Test for alkaloids

200 mg leaf material was boiled in 10 ml methanol and filtered. 1% HCl was added followed by 6 drops of Dragendorff reagent, and brownish-red precipitate was taken as evidence for the presence of alkaloids.

2.5.2. Test for Terpenoids

To 200 mg plant material, 2 ml of chloroform ($CHCl_3$) and 3 ml of concentrated sulphuric acid (H_2SO_4) were carefully added. A reddish-brown coloration signified the presence of terpenoids.

2.5.3. Test for Flavonoids

To the aqueous filtrate 5 ml of dilute ammonia solution was added, followed by concentrated H_2SO_4. A yellow coloration indicated the presence of flavonoids.

2.5.4. Test for Saponins

5 ml distilled water was added to 200 mg plant material. 0.5 ml filtrate was diluted to 5 ml with distilled water and shaken vigorously for 2 minutes. Formation of stable foam indicates the presence of saponins.

2.5.5 Test for Phenols

A few drops of ferric chloride solution were added to 2ml of the extract in a watch glass; the appearance of bluish green color indicated the presence of phenol.

3. Results and Discussion

3.1. Ovicidal activity

The hatchability of eggs of *H. armigera* at 96 h old were tested with topical application of crude methanol, acetone and aqueous leaf extracts of *Atalantia monophylla* at different concentrations viz.0.1. 0.2, 0.6, 0.8 and 1.0%. From the Table.1, it is clear that the methanol leaf extract at higher concentration (1.0%) show highest percentage of ovicidal activity (44±2.0). An increase in the concentration of methanol leaf extract level from 0.1% to 0.8% progressive increase in ovicidal activity was observed (08±0.0 to 23±0.1) just after 24 hours of treatment. Ovicidal activity also increases with the time period (16±1.2. to 44±2.0) after 96 hour of treatment. It indicates that the mobility of leaf extract through egg coat increases with time

Acetone leaf extracts tested at 1.0% concentration revealed maximum ovicidal effect after 96 hours (49±0.3) of treatment. The level of extract concentration and after treatment period has shown correlative effect with ovicidal activity (Table 2). Aqueous leaf extract has also proved significant ovicidal effect at higher concentration (Table 3).

Among the different leaf extract tested for ovicidal activity, acetone leaf extract was comparatively superior. These results correlates with the earlier works [11, 12, 13].

3.2. Phytochemical analysis

The results of the phytochemical screening, as shown in Table 4 below revealed positive for terpenoids, phenols and saponins in all solvents.

Alkaloids, flavonoids and terpenoids were positive in acetone and methanol extracts and negative in water. Phenols and saponins were present in methanol, acetone and water. Basu and Basu [14] reported the presence of alkaloid N-methylbicycloatalaphylline in *Atalantia monophylla.* Presence of alkaloids, coumarin, quinone and terpenoids has reported in member of Rutaceae [15].

Table 1: Effect of different concentrations of *A.monophylla* methanol leaf extracts on ovicidal activity of *H. armigera.*

Observation (hrs)	Ovicidal Activity (%)						
	Treatment Concentration (%) of leaf extract						
	0.1	0.2	0.4	0.6	0.8	1.0	Control
24	08±0.0	08±0.0	10±0.2	13±1.0	13±0.1	23±0.1	08±1.0
48	16±1.2	18±2.2	19±1.3	19±0.5	34±1.0	44±2.0	15±1.2
96	16±1.2	18±2.2	19±1.3	19±0.5	34±1.0	44±2.0	15±1.2

Values are mean of five replicates of three trials. ± = Standard deviation

Table 2: Effect of different concentrations of *A.monophylla* acetone leaf extracts on ovicidal activity of *H. armigera.*

Observation (hrs)	Ovicidal Activity (%)						
	Treatment Concentration (%) of leaf extract						
	0.1	0.2	0.4	0.6	0.8	1.0	Control
24	09±0.2	10±0.2	12±0.1	15±1.0	28±0.4	33±2.1	08±1.0
48	16±1.2	16±2.2	18±1.0	22±0.0	41±1.2	48±0.0	15±1.2
96	16±1.2	16±2.2	18±1.0	22±0.0	44±1.2	49±0.3	15±1.2

Table 3: Effect of different concentrations of *A. monophylla* aqueous leaf extracts on ovicidal activity of *H.armigera.*

Observation (hrs)	Ovicidal Activity (%)						
	Treatment Concentration (%) of leaf extract						
	0.1	0.2	0.4	0.6	0.8	1.0	Control
24	09±0.2	09±0.2	10±0.1	10±1.5	12±0.4	22±2.1	08±1.0
48	16±1.2	16±0.0	18±1.0	21±0.4	33±0.0	40±1.0	15±1.2
96	16±1.2	16±0.0	18±0.0	21±0.4	34±0.0	41±0.0	15±1.2

Table 4: Qualitative phytochemical test for different solvent leaf extract of *A. monophylla*

Phytochemical constituents	Methanol	Acetone	Water
Alkaloids	+	+	-
Terpenoids	+	+	-
Flavonoids	+	+	-
Saponins	+	+	+
Phenols	+	+	+

(+ = Present, - = Absent).

4. Conclusion

The acetone extract of *A. monophylla* at 1.0% concentration demonstrated highest ovicidal activity against *H.armigera* followed by methanol and water. The preliminary phytochemical analysis revealed the presence secondary metabolites viz. terpenoids, phenolics and saponins in *A. monophylla*.

Hence it is inferred that *A. monophylla* can be used further for details phytochemical investigation to develop a new botanical formulation for the management of *H. armigera.*

5. Acknowledgement

We are thankful to Science Instrumentation Facility Centre (DST-FIST, SR/FST/College-281), Devchand College, Arjunnagar, Kolhapur-591237, Maharashtra, India for providing all necessary facilities.

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