International Journal of Entomology Research
ISSN: 2455-4758
Impact Factor: RJIF 5.24
www.entomologyjournals.com
Volume 2; Issue 6; November 2017; Page No. 67-70

Effect of leaf extract of *Andrographis paniculata* against cotton pest spotted bollworm *Earias vittella*

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Abstract
The effects of methanolic extracts of *Andrographis paniculata* on the oviposition behavior, hatchability of eggs, larva, pupa and adult development of *Earias vittella* were investigated under laboratory conditions. Treatments included with difference concentrations of extract to compare with untreated control. Egg were dipped in different concentration (2,4 and 6) of extract with poor hatching. Larva fed on an extract sprayed cotton fruit slices. Adult fed on an extract containing 10% sucrose diet. There was poor egg laying.

Keywords: *Andrographis paniculata*, *Earias vittella*, oviposition, larva, pupa and adult

1. Introduction
Cotton is natural vegetable fibre of great economic important as a raw material for cloth. Its widespread use is largely due to the ease with which its fibres are spun info yarns. Cotton's strength, absorbency and capacity to be washed and dyed also make it adaptable to a considerable variety of textile products. Cotton producing areas in India are spread throughout the country. Punjab, Haryana, Rajasthan, Maharastra, Gujarat, Madhya Pradesh, Andhra Pradesh, Tamilnadu and Karnataka are the major cotton producing states.

Cotton is produced by small trees and shrubs which bears the botanical name Gossypium. One or two weeks after sowing shoots appears and 50 to 80 days later flowering begins. First buds are formed after three weeks blossoms appear after blossoming the petals fall of and the off spring or the boll develops. The boll is divided by partition in to 3-5 sections containing seeds, fibre grows on the seeds.

Spotted bollworm *Earias vittella* is an important pest of cotton. Larvae infest tender shoots, flower and fruits. They bore the tender shoot and exhibits dry shoot symptom. It limits fruit production by premature fruit drop. Larva bore into the tender shoots, flower buds and fruits. As a result, the shoot dry and flower buds and fruits drop prematurely. Fruits remaining on the plants are deformed and often show exit holes of the larvae. (Vevai, 1969) [12].

Earias vittella is an important pest of cotton in India, south east, Asia and Africa (Reed 1974) [8] (Saini and Singh, 1999) [9]. *Earias vittella* pest is active almost throughout the year on its different host plants. In cotton crop its initial attack is noticed in June and July. The attack on the bolls is generally higher than buds. Maximum infestation is recorded during August and September (Dhawan and Simwac, 1992) [2]. As a result of attack the quality and quantity of the cotton is reduced. A single larva can destroy several buds and bolls in its life. A single female may lays up to 300 to 400 eggs. Egg incubation period 3 to 9 days, larval period is 9-20 days. The pupal period is 2 weeks in summer, 3 weeks in winter. In summer, total life cycle completes within a month.

2. Materials and Methods
Collection of Test Insect
Earias vittella were collected the infested fruit of cotton plant from cotton field at Belukurichi, Namakkal District, Tamilnadu, India.

Collection of Plant Materials
The plants *Andrographis paniculata* (Sriyanangai in Tamil) were collected form in and around Belukurichi, Namakkal District, Tamil Nadu, India.

Preparation of Plant Extract
Collect the *Andrographis paniculata* plant dried and powder in a mixer grinder and stored the air tight container. About 500gm of the powdered material was subjected to soxhlation and exhaustively extracted with 90% Methanol for 48 hours. This extracts were concentrated in a rotary evaporator finally dark brown coloured material was obtained. It was stored in the refrigerator.

Rearing of Test Insect
Earias vittella egg clusters collected from the cotton field and kept in petriplates over the filter papers. Newly hatched larval were transferred to cotton plant stem dipped in water with glass bottles fresh cotton were provided daily to the larval till pupation. Pupal were collected from the glass bottles and kept in glass jars over a piece of filter paper. In each jar, resting place was provided to the newly hatched, adults for normal expansion of wings. The adults were provided with sugar solution (10%) soaked in cotton swabs and some shoots of cotton.

Experimental Design
The fruit and shoot borer of cotton *Earias vittella* egg, larva,

Pupa and adult were divided into 2 groups. Each groups comprising of 20 insects.

Group I: Server as normal control *Earias vittella's* egg, larva, pupa and adult were give only normal feed.

Group II: This group was treated with leaf extract of *Andrographis paniculata*. *Earias vittella* egg, Larva, Pupa and adult were treated with different concentration of leaf extract of *Andrographis paniculata*.

Effect of Leaf Extract of *Andrographis paniculata* on the Eggs of *Earias vittella*

Eggs of *Earias vittella* were collected from the laboratory stock culture. The eggs were counted and placed in a petriplates covering the month with muslin clothe. Leaf extract of *Andrographis paniculata* at 2%, 4%, 6% concentration was directly sprayed on the eggs using an atomiser. This experiment was replicated thrice. Hatchability of egg was recorded at the end of 72 hours of treatment by counting the number of emerged ones and under developed eggs using hand lens.

Effect of Leaf Extract of *Andrographis paniculata* on the I, II, III, IV and V Instars of *Earias vittella*

Laboratory reared various level stages (I, II, III, IV & V) of *Earias vittella's* were collected and they introduced into small slices of cotton fruits and they were placed in a Petridis with a wet filter paper and mouth of the Petridis were covered with a muslin cloth. Extract of *Andrographis paniculata* in different concentration 3%, 10% for I and II instars and 6%, 10% for III and IV instars and 9%, 10% for V instars larval respectively, were sprayed directly over the cotton fruit slices by using an atomizer. The experiment was replicated thrice. Sprayed with our study material *Andrographis paniculata* with a time interval of 48 hours. Mortality count was recorded at the end of 96 hours of treatments by counting the number of dead ones. The animals did not exhibit any movement by the touch of the camel brush is conformed as dead ones. Mortality and delayed in Pupation were recorded.

Effect of Leaf Extract of *Andrographis paniculata* on the Pupa of *Earias vittella*

Laboratory reared pupa of the *Earias vittella* were collected from the stock culture. Treated with our study materials *Andrographis paniculata* sprayed directly over the pupa at 2% concentration to 1 day old pupa 4% concentration to 2 day old pupa 6% concentration to 3 day old pupa, 8% concentration to 4 day old pupa and 10% concentration to 5 day old pupa. During the investigation period, pupal mortality, non emergence, delay emergence were noted and recorded.

Effect of Leaf Extract of *Andrographis paniculata* on the Adult Insect of *Earias vittella*

Laboratory reared male and female insect of *Earias vittella* were housed in mating jar. They were provided with 10% sucrose solution and add with 10% concentration of *Andrographis paniculata*. They were observed for its egg laying and adult deformities were recorded.

Percent mortality of the test insects was calculated and corrected by using Abbott's formula (Abbott's 1925) [1].

$$\text{Corrected Mortality} = \frac{\text{observed mortality in treatments} - \text{observed mortality in control}}{100 - \text{control mortality}} \times 100$$

$$\text{Percentage of mortality} = \frac{\text{Number of dead insect}}{\text{Number of Larval introduced}} \times 100$$

Effect of Leaf Extract of *Andrographis paniculata* on the total body protein in the Larval, Pupal and Adult Stages of *Earias vittella*:

Five larvae from each set were taken. The larvae were homogenized in phosphate buffer and centrifuged at 2000 rpm for 15 min. supernatant was taken and 2.5 ml of trichloroacetic acid was added. It was centrifuged at 2000 rpm for 15 min again and supernatant was discarded. Then the protein is estimated by Lowry *et.al.,* (1951) [7] method.

Materials

1. Complex reagent 2% Na_2CO_3 + 1% $CuSO_4$ + 2% KNa_4
2. Prepare 2N NaOH
3. Folin reagent
4. Bovin Serum albumin

Standards: 1ml bovin serum albumin was dissolved in 1ml of distilled water.

Method

To 0.1 ml of sample add 0.1 ml of 2N NaOH heating of 100ºC (water boiling). Cool at room temperature and add 1ml of complex solution let the room temperature for 10 min. Add 0.1 ml of folin reagent and well mixed. After 30-60 min. determine the optical density (OD) of the sample and standards. Calculate the initial control larval protein value and final treated larval protein value.

$$= \frac{\text{OD of unknown sample}}{\text{OD of known sample}} \times \text{Concentration of Standard} \times \frac{1000}{\text{Weight of tissue}}$$

3. Experimental Result

Studies were undertaken to know the effort of leaf extract of *Andrographis paniculata* on fruit and shoot borer *Earias vittella*. The results obtained are presented below.

Total Number of Egg Laid

Group I control insects eggs laid was 95 percent. Group II insect treated with leaf extract of *Andrographis paniculata* at 2% dosage has reduced the oviposition to 68.75%. It was found to be a significant reduction in the oviposition of Group II insect when compared to Group I, 4% dosage has reduced the oviposition to 43.35%. It was found to be a significant reduction in the oviposition of group I insect when compared to group I treated insect 6% dosage has reduced the oviposition to 29.84%.

Egg Hatchability Percentage

The percentage of egg hatchability in Group I control insect was 92.78%. But Group II insects treated with leaf extract *Andrographis paniculata* at 6% dosage it was 23.7%.

Effect of Leaf Extract of *Andrographis paniculata* on the Larval Mortality of I, II, III, IV, V instars Larval of *Earias vittella*

The percentage of mortality of Group II insects, I instars larva stage of control insects mortality was 2.85% but treated with 1%, 5% and 10% *Andrographis paniculata* causes a mortality of 34.76%, 68.76% and 82.69%. II instars larva stage of control insects mortality was 2.60% but treated with 1%, 5% and 10% *Andrographis paniculata* causes a mortality of 31.71%, 64.99% and 86.40%. III instars larva stage of control insects mortality was 2.58% but treated with 1%, 5% and 10% *Andrographis paniculata* causes a mortality of 29.20%, 64.85% and 87.61%. IV instars larva stage of control insects mortality was 2.37% but treated with 1%, 5% and 10% *Andrographis paniculata* causes a mortality of 29.00%, 61.58% and 87.00%. V instars larva stage of control insects mortality was 1.82% but treated with 1%, 5% and 10% *Andrographis paniculata* causes a mortality of 28.61%, 60.71% and 86.21%.

Effect of Leaf Extract of *Andrographis paniculata* on the Pupal Mortality of *Earias vittella*

Group I insect did not come across any deformities. But Group II insects treated with leaf extract *Andrographis paniculata* causes 76.90% deformities.

Effect of Leaf Extract of *Andrographis paniculata* on the Adult Mortality of *Earias vittella*

Group I insect did not come across any deformities. But Group II insects treated with leaf extract *Andrographis paniculata* causes 72.92% deformities.

Effect of Leaf Extract of *Andrographis paniculata* on the total body protein in the Larval, Pupal and Adult Stages of *Earias vittella*

Effect on Larval Stages

Group II *Andrographis paniculata* 5% and 10% treated second instar larval 3.00 ± 0.31mg/g and 3.23 ± 0.35 mg/g reduction in the total body protein compared to group I control. In group II third instar larval treated with *Andrographis paniculata* 5% and 10% reduction in the total body protein 4.21 ± 0.20 mg/g and 4.35 ± 0.25 mg/g. In group II fourth instar larval treated with *Andrographis paniculata* 5% and 10% reduction in the total body protein 6.20 ± 0.10 mg/g and 6.30 ± 0.15 mg/g. In group II fifth instar larval treated with *Andrographis paniculata* 5% and 10% reduction in the total body protein 9.00 ± 0.30 mg/g and 9.75 ± 0.53 mg/g.

Effect on Pupal Stage

Group II Pupa treated with *Andrographis paniculata* 5% and 10% reduction in the total body protein 15.30 ± 0.20 mg/g and 15.75 ± 0.25 mg/g.

Effect on Adult Stage

Group II adult treated with *Andrographis paniculata* 5% and 10% reduction in the total body protein 13.15 ± 0.20 mg/g and 13.20 ± 0.45 mg/g.

Table 1: Effects of methanolic leaf extract of *Andrographis paniculata* on larval mortality of I,II,III,IV and V instars of *Earias vittella*

Experimental Group	Dose	Mortality (%)				
		I - Instar	II - Instar	III – Instar	IV – Instar	V – Instar
Group I control	No treatment	2.85	2.60	2.58	2.37	1.82
Group II Treated with *Andrographis paniculata*	1%	34.76	31.71	29.70	29.00	28.61
	5%	68.76	64.99	64.85	61.58	60.71
	10%	82.69	86.40	87.61	87.00	86.21

Table 2: Effects of methanolic leaf extract of *Andrographis paniculata* on the total body proteins in the larval, pupal and adult stages of *Earias vittella*

Experimental Group	Dose	Total Body Protein					
		II - Instar	III - Instar	IV – Instar	V – Instar	Pupa	Adult
Group I control	No treatment	3.64 ± 0.28	4.75 ± 0.43	6.87 ± 0.55	9.83 ± 0.40	16.42 ± 0.70	13.43 ± 1.25
Group II Treated with *Andrographis paniculata*	5%	3.00 ± 0.31	4.21 ± 0.20	6.20 ± 0.10	9.00 ± 0.30	15.30 ± 0.20	13.15 ± 0.20
	10%	3.23 ± 0.35	4.35 ± 0.25	6.30 ± 0.15	9.75 ± 0.53	15.75 ± 0.25	13.25 ± 0.45

4. Discussion

Earias vittella eggs as the concentration gradually increased from 2 to 6%. *Andrographis paniculata* caused a greater reduction in the number of eggs laid compared with the control different concentrations of ethanolic extract of *Andrographis paniculata* caused a greater reductions in the number of Larva, Pupa and Adult. Repellent activity of neem against oviposition by lepidopterous pests has also been reported for *Spodoptera litura* (Joshi and Sitaramaiah, 1979) [5], C*raphalicrocis medinalis* (Saxena *et al.*, 1981) [11]. *Earias vittella* adults were exposed to the extract treated glass surface without actual contact by the moths there was a reduction in egg laying which is a clear indication of the repellent action of *Andrographis paniculata* volatiles were reported against *Spodoptera frugipoqda* (Hellpap and Mercado, 1986) [3] and *Helicoverpa armigesa* (Saxena and Rembold, 1984) [10] ethanolic extracts of both botanicals at different doses caused significant reduction in egg hatching, larva, pupa and adult development of *Earias vittella*. The ovicidal action of neem extracts has also been reported against plutella xylostella (Loke *et al.*, 1992) [6] and *Helicoverpa armigesa* (Jeyakumar and Gupta, 1999) [4].

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