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Research Article

Larvicidal and antifeedant activity of some indigenous plants of Meghalaya against 4th instar *Helicoverpa armigera* (Hübner) larvae

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Abstract: In the present study, seven indigenous, widely distributed plants of Meghalaya, namely, *Pinus kesiya* Royle (Pinaceae), *Lantana camara* Linn. (Verbenaceae), *Litsea cubeba* Lour. (Lauraceae), *Gaultheria fragrantissima* Wall. (Ericaceae), *Mikania micrantha* Kunth.(Asteraceae), *Ambrosia artemisiifolia* Linn.(Asteraceae) and *Eupatorium riparium* Regel (Asteraceae) were screened for their larvicidal and antifeedant activity against fourth instar larvae of the cotton bollworm, *Helicoverpa armigera* (Hübner) under laboratory conditions. The crude extracts of all the seven plants demonstrated a dose dependent increase in bioactivity. However the bioactivity of four plants namely, *L. camara, G. fragrantissima, L. cubeba* and *P. kesiya* was significantly higher ($p \leq 0.05$) than the negative (solvent) control and extracts of *A. artemisiifolia, E. riparium* and *M. micarantha*. Methanol extract of *L. camara* caused highest oral toxicity with larval mortality ranging between 27.77% and 53.33% across the test concentration (0.25%, 0.5% and 1% w/v) while extract of *G. fragrantissima* demonstrated the highest feeding deterrence with reduction in larval feeding by50.92% and 70.61% at 0.1% and 0.5% respectively. Crude extract of *L. cubeba* leaves demonstrated high oral toxicity and feeding deterrence while extract of the needles of *P. kesiya* showed moderate level of oral toxicity as well as feeding deterrence at the highest tested concentration. Phytochemical analysis of the extracts of these four plants, revealed the presence of five different classes of phytocompounds each of which is known to have deleterious effect on insect pests.Thus it may be concluded that four out of the seven plants possess insecticidal property and can be further investigated for the development of a potent botanical insecticide.

Keywords: plant extract, oral toxicity, antifeedant activity, *Helicoverpa armigera*, Meghalaya

Introduction

Helicoverpa armigera Hübner (Lepidoptera: Noctuidae) is a polyphagous migratory noctuid which is widespread in Asia, Africa and Oceania (Lammers and Macleod, 2007). It is known to cause serious damage to hundreds of economically important crops all over the world (Setiawati *et al*., 2000; Fakrudin *et al*., 2004). In India it is reported to be feeding on 182 plant species across 47 families (Manjunath *et al*., 1985) and causes an annual loss of about Rs. 2,000 crores (Ignacimuthu and Jayaraj, 2003).

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Fifty percent of all insecticides used in India and China are to control *H. armigera* alone (Lammers and Macleod, 2007) 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 in various parts of the world (McCaffery, 1998; Chaturvedi, 2007; Yang *et al*., 2013).Thus alternatives to the synthetic pesticides are being sought.

The search for alternatives to synthetic pesticides has focused the interest of the pest managers on plant derived pest control agents. Plant-based pesticides or botanicals have many advantages: firstly, they have multifarious control mechanisms against pests (Sivagnaname and Kalyanasundaram, 2004) which reduces the possibility of the development of resistance in pests (Liu *et al*., 2000); secondly, they are target-specific and hence not harmful to humans and beneficial insects; and lastly, they are not persistent in nature and hence environment friendly (Shaalan, 2005).

In the present investigation an attempt has been made to screen seven widely distributed plants of Meghalaya, 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). The effect of many different plants and their extracts on *H. armigera* has been studied by several authors (Pandey *et al*., 1983; Jotwani and Srivastava, 1984; Hongo and Karel, 1986; Sahayaraj, 1998; Sundararajan and Kumuthakalavalli 2001; Koul *et al*., 2002; Kathuria and Kaushik, 2005; Ramya *et al*., 2008; Wambua *et al*., 2011; Jeyashankar *et al*., 2012; Arivoli and Tennyson, 2013). While extracts of certain plants like *Ocimum basilicum, Gynandropsis gynandra, Acorus calamus, Lantana camara,* and *Toddalia asiatica* demonstrated larvicidal effect on *H. armigera* (Pandey *et al*., 1983; Sundararajan and Kumuthakalavalli, 2001), others like neem seed kernel extract were seen to have indirect effects like causing larval-pupal intermediaries and abnormal adults (Jotwani and Srivastava, 1984) and feeding deterrency (Hongo and Karel, 1986). Majority of the plants tested against different larval instars of *H. armige*ra have been reported to demonstrate antifeedant properties (Sahayaraj, 1998; Koul *et al*., 2002; Kathuria and Kaushik, 2005; Ramya *et al*., 2008; Wambua *et al*., 2011; Jeyashankar *et al*., 2012; Arivoli and Tennyson, 2013).

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 *Lantana camara, Pinus kesiya, Litsea cubeba, Gaultheria fragrantissima, Mikania micrantha, Ambrosia artemisiifolia and Eupatorium riparium,* which have a wide distribution in the state of Meghalaya and find application in medicinal practices of the local tribal population (Neogi *et al*., 1989; Chhetri, 2008; Hynniewta and Kumar, 2008; Kayang *et al*., 2008; Sinha *et al*., 2008; Sohkhlet, 2014). The present study is aimed at determining the oral toxicity and antifeedant activity of the above mentioned plants against fourth instar larvae of *H. armigera* (Hübner).

Materials and Methods

Collection of plants:

The seven plants selected for this study were collected from in and around Shillong city in Meghalaya. 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 (Table 1). The samples were generally collected during the flowering and fruiting stage of the plants except *P. kesiya*. In case of *P. kesiya*, samples were collected from young plants aged between 10 to 12 years, mainly during spring and summer seasons when fresh needles emerged. The collected plants were identified by Dr. P. B. Gurung, Department of Botany, N. E. H. U., Shillong.

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Table 1 Details of the plant parts used and the collection sites of the seven plants selected for the study.

Sl. no.	Scientific name	Local Name	Site of Collection	Plant Part Used
1	*Ambrosia artemisiifolia* Linn. (Asteraceae)	Jaiawlong/kynbat Japan rit	N. E. H. U. campus, Shillong	Aerial parts
2	*Eupatorium riparium* Regel (Asteraceae)	Kynbat latnaiong	N. E. H. U. campus, Shillong	Leaves
3	*Gaultheria fragrantissima* Wall. (Ericaceae)	Sla thynrait	Mylliem, Upper Shillong.	Leaves
4	*Lantana camara* Linn. (Verbenaceae)	Soh Pyngkhlieh	N. E. H. U. campus	Aerial parts
5	*Litsea cubeba* (Lour.) Pers. (Lauraceae)	Dieng mosu/Dieng-si-ing	N. E. H. U. campus	Leaves
6	*Mikania micrantha* Kunth. (Asteraceae)	-	N. E. H. U. campus	Aerial parts
7	*Pinus kesiya* Royle (Pinaceae)	Dieng kseh	N. E. H. U. campus	Needles and tender branches.

Preparation of plant extracts:

The plants were brought to the laboratory immediately after collection and washed with tap water thoroughly followed by a final rinse with dechlorinated water, following which, they were shade dried at room temperature (21 ± 2 °C) for 48-72 hours, depending on the plant. The dried plants were ground to coarse powder (~2mm) using an electric blender. The crude extracts were prepared using standard protocol (Harborne, 1998; Houghton and Raman, 1998; Kathuria and Kaushik, 2005; Handa *et al*., 2008; Deepa and Remadevi, 2011). For the preparation of extracts, 250 g of each of the plant powders was extracted with 2.5 litres methanol using a Soxhlet apparatus for 48 hours. Prior to extraction with methanol, the plant material was defatted with petroleum ether. The extracts were taken to dryness under reduced pressure using a rotary-vacuum evaporator and stored in airtight screw capped borosil containers at -20 °C for future use. Prior to performance of a bioassay, a standard stock solution of 2.5% w/v concentration was prepared by dissolving 2.5 g of the extract in 10 mL acetone and volume was made up to 100 ml by adding deionized water. From the stock solution, 0.25%, 0.5% and 1% w/v concentration was prepared for ingestion toxicity test and 0.1%, 0.2% and 0.5% w/v concentration for feeding deterrence test. Final volume for each of the test concentrations was 20 ml.

Test insect:

A laboratory culture of *H. armigera* larvae was maintained on a chickpea based semi-synthetic diet as suggested by Singh and Rembold (1992) under laboratory conditions (21 ± 2 °C, 80 ± 5% R.H., and photoperiod of 12 L: 12 D). For the initial establishment of the colony in the laboratory, different instars of *H. armigera* larvae were collected from tomato crops grown in Mawiongsun village under Mawrynkneng tehsil in East Khasi Hills, District. The collected larvae were maintained on tomato leaves and fruits under laboratory conditions (21 ± 2 °C, 80 ± 5% R. H. and photoperiod of 12 L: 12 D) in individual containers to prevent cannibalism and contamination until pupation. Pupae were transferred to clean containers with sterilized filter paper to facilitate moth emergence. Upon adult emergence, the male and female moths were paired and two pairs were released into individual mating chambers (2.5x1.6 feet). The adults were fed on a diet of 10% honey solution and provided with cotton strips as oviposition medium (Kaushik and Kathuria, 2004). From the first generation onwards, the laboratory colony was maintained on a chickpea based semi-synthetic diet. From the cultures, newly molted one-day old IV instar larvae were used for the bioassays.

Ingestion toxicity bioassay:

The larvicidal activity of plants was studied by oral application of the extracts through leaf dip method (Ramya *et al*., 2008). Freshly collected tomato leaves were individually dipped in the three different concentrations (0.25%, 0.5% and 1% w/v) of each of the extracts and air dried. A single treated leaf was kept in a petri plate lined with moist filter paper and a single 6 hour starved fourth instar *H. armigera* larvae was introduced into the petri plate. Leaves treated with acetone were used as negative control while those treated with 100 ppm of Alphamethrin 10% EC (trade name: GEM)

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were used as positive control. Alphamethrin 10% EC is a pyrethroid insecticide which demonstrates both contact and stomach toxicity against a wide range of insect pests (Indofil, 2016). Larval mortality was recorded after 24 hours of exposure. A total of 10 larvae were individually exposed to each treatment and each treatment was replicated thrice. The total number of subjects per treatment was 30 larvae. The mortality data were represented as corrected mortality using Abbott's correction (Abbot, 1925).

$Ma\% = [(M_t - M_c) / (100 - M_c)] \times 100$

Ma% = corrected mortality(%), M_t = mortality in treatment(%), M_c = mortality in control(%).

Feeding deterrence bioassay:

The antifeedant activity of crude extracts was assayed using leaf disc method (Rani and Rajasekharreddy, 2009; Li *et al*., 2014). Discs of size 2.75cm^2 were punched from freshly collected tomato leaves and treated on each side with 15 µl of the test solution emulsified with 0.1% Triton X-100. The extracts were tested at three different concentrations-0.1%, 0.2% and 0.5% w/v. Leaf discs treated with acetone solution and emulsifier (0.1%) were used as control. The leaf discs were air dried and arranged in a petri plate with one treated and one control leaf disc per plate. A fourth instar larva of *H. armigera* was then introduced at the center of the petri plate, such that it was equidistant from the treated and the control discs. The experiment was thus conducted with one larva per petri plate with ten larvae per treatment and each treatment was replicated three times. After 6 hours, the leaf discs were removed and the area consumed by the larvae was measured using a graph sheet method. The feeding deterrence index was calculated by using the formula given by Bomford and Isman (1996):

$$FDI = \frac{C-T}{C+T} \times 100$$

Where, C = area of consumption in the control; T = area of consumption in the treatment.

Phytochemical analysis

The presence of different classes of phytochemicals in the plants demonstrating high oral toxicity and antifeedant activity was investigated qualitatively using standard procedures as described by Trease and Evans (1989), Sofowara (1993) and Harborne (1998).

Alkaloids

0.5 gm of the methanol extract was mixed with 8 ml of 1 % HCl, warmed and filtered. In a test tube, 2 ml of the filtrate was taken and a few drops of Dragendroffs reagent (solution of Potassium Bismuth Iodide) was added along the side of the test tube. Formation of red precipitate indicated presence of alkaloids.

Flavonoids

0.5 gm of the extract was shaken with petroleum ether to remove the lipid layer. The defatted residue was dissolved in 20 ml of 80 % ethanol and filtered. Three ml of the filtrate was mixed with 4 ml of 1 % potassium hydroxide solution in a test tube and the colour was observed. A dark yellow color indicated the presence of flavonoids.

Phenols

0.5 gm of the methanol extract was dissolved in 5 ml distilled water and then few drops of neutral 5% ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds.

Phytosterols

To 2 ml of the extract, 2 ml of chloroform was added followed by 2 ml of concentrated sulphuric acid. Formation of red colour in the chloroform layer indicated the presence of steroids.

Saponins

0.5 gm of the extract was dissolved in distilled water in a test tube and heated over a boiling water bath. The test tube was allowed to cool and then shaken vigorously. Formation of persistent froth indicated presence of saponins.

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Tannins
0.5 gm of the extract was dissolved in 20 ml distilled water in a test tube and boiled. The solution was filtered and 1% aqueous ferric chloride was added to the filtrate. The appearance of intense green, purple, blue or black colour indicated the presence of tannins.

Terpenoids
5ml of the extract was mixed in 2 ml of chloroform, followed by the careful addition of 3 ml of concentrated sulphuric acid. A layer of reddish brown colouration at the interface indicated the presence of terpenoids.

Data analysis
The data obtained from the two bioassays were subjected to arcsine transformation prior to statistical analysis. The transformed data were then statistically analysed by one-way ANOVA. Separation of means and comparison between the different treatments was performed by Tukey's test at $P \leq 0.05$. SPSS version 20 was used for the analysis.

Results

Ingestion toxicity bioassay
The larvicidal activity of methanolic extract of the seven plant species is presented in Table 2. All the seven plants demonstrated a dose dependent increase in oral toxicity, with percentage mortality of fourth instar larvae of *H. armigera* being highest at test concentration of 1% w/v. When tested at the concentration of 0.25%, methanol extract of all the seven plants demonstrated an average mortality of 15.78% which was statistically similar ($p > 0.05$) to the mortality rate of the larvae in the negative (solvent) control. However, at concentration of 0.5% and 1% w/v, the crude extracts of *L.camara*, *L.cubeba* and *P.kesiya* caused significantly higher mortality ($p \leq 0.000$) than the negative control. The larvicidal activity demonstrated by the extract of aerial parts of *Lantana camara* against fourth instar larvae of *H. armigera*, was the highest amongst all the seven plants with percent corrected mortality ranging from 27.77% to 53.33% across the test concentration. Its larvicidal activity was significantly higher than the negative control and the other plants ($p \leq 0.05$) except *L. cubeba* ($p = 0.672$) and *P.kesiya* ($p = 0.315$). The methanol extract of the leaves of *Litsea cubeba* demonstrated the second highest oral toxicity against fourth instar larvae of *H. armigera*, with corrected larval mortality ranging between 20.37% and 51.48% across the test concentration. Of the remaining five plants, extract of *Pinus kesiya* caused 37.77% larval mortality at the highest concentration of 1% w/v and it was significantly higher ($p=0.014$) than the larval mortality in negative control, thereby making it the third best plant after *L. camara* and *L. cubeba* in terms of oral toxicity against fourth instar larvae of *H. armigera*. However, it is to be noted that the synthetic insecticide, Alphamethrin 10% EC, which was used as a reference/positive control in this bioassay, caused 100% larval mortality within 24 hours of exposure and its activity was significantly higher ($p \leq 0.000$) than the activity of the plant extracts.

Feeding deterrence bioassay
The antifeedant activity of crude extracts of the selected plants was studied at three different concentrations. The feeding deterrence activity of the plants was assessed on the basis of the feeding deterrence index (FDI). Higher antifeedant/ feeding deterrence index indicates lower feeding by the test organism. All the seven plants demonstrated dose dependent increase in feeding deterrence but irrespective of the test concentration of the plant extracts, the antifeedance index of the negative (solvent) control was significantly lower ($p \leq 0.0001$) in comparison to that of the plants (Table 3). Of the seven plants, the crude extract of *G. fragrantissima* demonstrated the highest antifeedant activity, causing 50.92% to 70.61% reduction in feeding by the fourth instar larvae of *H. armigera*, across the test concentration and thus its FDI was significantly higher ($p \leq 0.05$) than the other six plants. Apart from *G. fragrantissima*, crude extract of *L. cubeba, P.*

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kesiya and *L. camara*, also caused high feeding deterrence, which was significantly higher than the remaining three plants with $p \leq 0.05$. While the FDI on exposure to *L. cubeba* extract was 27.17% to 56.78% across test concentrations, *P. kesiya* extract reduced larval feeding by 36.07% to 49.39%; and, *L. camara* extract reduced larval feeding in the range of 18.66 % to 40.73% across the test concentrations.

Phytochemical analysis:

Based on the outcome of the two bioassays, crude extract of four out of the seven plants demonstrated high larvicidal and antifeedant activity. Hence, qualitative analysis of the methanol extracts of these four plants, namely, *G. fragrantissima, L. camara, L. cubeba* and *P. kesiya*, was carried out for determination of the major phytochemical constituents present in them (Table 4). The outcome of the phytochemical analysis revealed that flavonoids, phenols and tannins were present in the extracts of all the four plants whereas alkaloids were detected only in extract of *P. kesiya*. Phytosterols were found in *L.camara* extract while terpenoids tested positive in the extracts of both *L. camara* and *L. cubeba*. Saponins were absent in the methanol extract of *L. camara* but present in the extracts of the other three plants.

Table 2 The larvicidal (oral) toxicity of the crude extracts of the seven selected plants against fourth instar *Helicoverpa armigera* larvae.

Plant Name	Concentration of Extract (% w/v)		
	0.25%	0.5%	1%
Ambrosia artemisiifolia	13.7 ± 5.48 ^{b}	13.70 ± 5.48^{def}	24.07 ± 5.25 ^{cd}
Eupatorium riparium	7.04 ± 6.12 ^{b}	7.04 ± 6.12^{ef}	13.33 ± 5.77 ^{de}
Gaultheria fragrantissima	13.33 ± 5.77^{b}	20.00 ± 10.00 ^{cde}	24.07 ± 5.25 ^{cd}
Lantana camara	27.77 ± 6.93^{b}	44.81 ± 5.01^{b}	53.33 ± 8.01 ^{b}
Litsea cubeba	20.37 ± 9.45^{b}	35.55 ± 3.85 ^{bcd}	51.48 ± 7.88^{b}
Mikania micrantha	7.50 ± 6.61^{b}	7.87 ± 6.85^{ef}	18.52 ± 6.41^{d}
Pinus kesiya	20.37 ± 9.45^{b}	34.44 ± 5.09^{bc}	37.77 ± 3.85 ^{bc}
Negative control	3.33 ± 5.77 ^{b}	3.33 ± 5.77 ^{f}	3.33 ± 5.77 ^{e}
Positive (reference)control	100^{a}	100^{a}	100^{a}

Mean ± SD represents mean percent corrected mortality of 3 replicates of 10 individuals each. Within columns, Means followed by the same alphabet do not differ significantly at 5% level of significance using Tukey's HSD test. Note: Chemical insecticide-Alphamethrin 10% EC at a concentration of 100 ppm was used as reference (positive) control while acetone was used as negative control.

Table 3 The antifeedant activity (feeding deterrence) of the crude extracts of each of the seven plants against fourth instar larvae of *Helicoverpa armigera.*

Plant Name	Concentration of Extract (%w/v)		
	0.1%	0.2%	0.5%
Ambrosia artemisiifolia	12.67 ± 1.44^{e}	12.42 ± 6.51^{cd}	17.12 ± 7.75^{d}
Eupatorium riparium	12.83 ± 0.83^{e}	15.8 ± 9.85^{bc}	17.31 ± 5.31^{d}
Gaultheria fragrantissima	52.27 ± 2.19^{a}	50.92 ± 11.21^{a}	70.61 ± 9.04^{a}
Lantana camara	18.66 ± 2.95^{de}	35.44 ± 6.83^{ab}	40.73 ± 8.55^{bc}
Litsea cubeba	27.17 ± 5.68^{c}	46.61 ± 7.16^{a}	56.78 ± 4.93^{ab}
Mikania micrantha	21.57 ± 1.31^{cd}	23.99 ± 6.03^{ab}	25.26 ± 5.92^{cd}
Pinus kesiya	36.07 ± 1.05^{b}	43.57 ± 6.7^{a}	49.39 ± 5.25^{b}
Negative (solvent) control	6.10 ± 1.21^{f}	5.50 ± 1.64^{d}	5.50 ± 1.64^{e}

Mean ± SD represents mean percent feeding deterrence of 3 replicates of 10 individuals each. Within columns, means followed by the same alphabet do not differ significantly at 5% level of significance using Tukey's HSD test. Acetone was used as negative control.

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Table 4 The major phytochemical constituents present in the methanol extracts of the four plants demonstrating insecticidal activity.

Classes of Phytochemicals	*G. fragrantissima*	*L. camara*	*L. cubeba*	*P. kesiya*
Alkaloids	-	-	-	+
Flavonoids	+	+	+	+
Phenols	+	+	+	+
Phytosterols	-	+	-	-
Saponins	+	-	+	+
Tannins	+	+	+	+
Terpenoids	-	+	+	-

(+) present; (-) absent.

Discussion

The ingestion toxicity bioassay revealed that larvicidal activity of the crude methanolic extract of the plants was much lower than that of the synthetic insecticide, Alphamethrin 10% EC. However, four out of the seven plants caused significantly higher ($p \leq 0.05$) larval mortality as well as feeding deterrence in comparison to the solvent (negative) control indicating potent insecticidal activity against the notorious pest, *H. armigera*.

The results of the present study indicated that at higher concentrations, *Lantana camara* L. could act both as a potent oral toxicant and feeding deterrent against *H. armigera* larvae, and this result is in agreement with the findings of Prasad and Roy (2011), who had concluded from their histopathological study that extracts of *L. camara* could act as stomach poison in addition to some antifeedant activity against fourth instar *H. armigera* larvae. In a study by Murugesan and co-workers (2012), it was reported that essential oil of *L. camara* at a concentration range of 2500-10000 ppm caused 20-50% larval mortality after 24 hours exposure against third instar larvae of teak defoliator, *Hyblaea puera* while in another study, aqueous crude extract of *L. camara* leaves at a concentration of 40% caused 100% mortality of fourth instar larvae of *Spodoptera litura* (Deshmukhe *et al.,* 2011). Both these studies found that larvicidal activity of *L. camara* increased with increase in its concentration which corroborated with our present findings. Also, several authors have studied the antifeedant activity of *L. camara* (Deka *et al.,* 1998; Ogendo *et al.,* 2003; Murugesan and Murugesh, 2009). In a study by Arivoli and Tennyson (2013), at a concentration of 1%, the ethyl acetate crude extract of *L. camara* showed 25-50% antifeedance against third instar larvae of *Spodoptera litura* while the hexane and dichloromethane extracts showed < 25% antifeedance. Our study indicated higher activity of methanolic extract of *L. camara*, causing 40.74% feeding deterrence against fourth instar *H. armigera* larvae, at a much lower concentration of 0.5% w/v. However, it may be noted that different test organisms were used in the investigations conducted by other authors and many studies have shown that even closely related insect species can show widely di□erent susceptibilities to the same extract or compound (Isman, 1993), which could be one of the reasons for the variation between the outcome of the present study and the previous studies.

Like *L. camara*, the crude extract of *L. cubeba* Pers. leaves showed significantly high ($p \leq 0.05$) larvicidal and antifeedant activity against fourth instar larvae of *H. armigera*. *Litsea cubeba* is an important medicinal plant which finds wide application in traditional Chinese medicine (Kong *et al*., 2015). Apart from having antifungal properties against several pathogens (Nor Azah and Susiarti, 1999), it has also been found to have strong repellent, contact and fumigant toxicity as well as deterrent effect against stored product pests (Liu *et al.,* 2007; Ko *et al.,* 2009). Also, its essential oil has been found to be moderately effective as a contact toxicant against third instar larvae of *Trichoplusia ni* (Jiang *et al.,* 2009) while Feng and co-workers (2012) reported that its ethanolic extract had strong feeding deterrence activity against third instar larvae of *Spodoptera litura*. To the best of our knowledge, the present study reports for the first time the toxicity of crude extract of *Litsea cubeba* against *Helicoverpa armigera* and a notable outcome is that at higher concentrations, it was moderately toxic (~43.51%) and demonstrated relatively high

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antifeedance (~51.69%) against *H. armigera*.

Another important finding from this study is the larvicidal and antifeedant activity displayed by *Pinus kesiya*. This is the first record of the insecticidal activity of needle extracts of *P. kesiya,* although, it finds application in the traditional pest management practices of the indigenous tribes of Meghalaya (Sinha *et al*., 2008; Sokhlet, 2014). The methanolic extract of *P. kesiya* needles, at the highest tested concentration, caused close to 40% larval mortality apart from deterring larval feeding by ~50%. Thus, the insecticidal activity demonstrated by *P. kesiya* needle extract against fourth instar *H. armigera* larvae was significantly ($p \leq 0.05$) higher than the negative (solvent) control. In fact, the FDI demonstrated by *P. kesiya* extract was comparable to the antifeedancy displayed by ethanolic and hexane extracts of *Eucalyptus camaldulensis* and *Tylophora indica against* fifth instar *H. armigera* (Kathuria and Kaushik, 2005). In a study by Kanis and co-workers (2009), a direct correlation was found between the lignin content of the acetone extracts of *Pinus caribaea* Morelet and their larvicidal activity against *Aedes aegypti*. Thus, although *P. kesiya* demonstrated moderate efficacy against *H. armigera* larvae in the present study, it may be a good candidate for future research.

During this study, the outcome of feeding deterrence bioassay was very encouraging with crude extract of *G. fragrantissima* demonstrating stronger antifeedant activity than plants like *Tephrosia vogelii* Hook (Leguminosae) and *Solanum pseudocapsicum* (Solanaceae) which have been reported to show promising antifeedant activity against larval stages of *H. armigera* by Jeyashankar and co-workers (2012) and Arivoli and Tennyson (2013), respectively. Although no earlier reports on the insecticidal activity of crude extracts of *G. fragrantissima* were found but several authors have studied the larvicidal, pupicidal, antifeedant and repellent activities of the essential oil of *Gaultheria* species (Senthilkumar and Venkatesalu, 2012; Ranyaphi *et al.*, 2012; Jeyasankar, 2012; Palanimuthu *et al.*, 2014). *Gaultheria fragrantissima* is rich in essential oil which is a constituent of several insecticidal and insect repellent preparations (Ranyaphi *et al.*, 2012) and therefore future investigation on its bioactivity could enable the development of an effective antifeedant against larval stages of polyphagous pests like *H. armigera.*

An important finding of the present study is that three out of four plants demonstrating high antifeedant activity also caused maximum larval mortality. Similar findings have been reported by several authors (Chen *et al*., 1996; Koul *et al.*, 2004; Ling *et al*., 2008). According to a study by Lingathurai and co-workers (2011), chloroform extract of *Acalypha fruticosa* Forssk leaves demonstrated maximum antifeedance and oral toxicity against third instar larvae of *Plutella xylostella* L.; the authors attributed the two different modes of action of the extract to the presence of five different phytochemical groups namely, terpenoids, tannins, coumarins, anthraquinones and saponins. In the present study too, the qualitative analysis revealed the presence of phytochemical groups like phenols, flavonoids, tannins, terpenoids, saponins and alkaloids in the methanol extract of *G. fragrantissima, L. camara, L. cubeba and P. kesiya*.

The insecticidal activity of plants is attributed to the presence of various phytochemical groups (Kabaru and Gichia, 2001) and the occurrence of more than one major class of phytocompounds is responsible for the different modes of action of plant extracts against the target pests (Park *et al.,* 2002; Lingathurai *et al*., 2011). The extract of the four plants, namely, *G. fragrantissima, L. camara, L. cubeba and P. kesiya*, tested positive for phenolic compounds, flavonoids and tannins. All three groups of phytocompounds have been reported to affect herbivorous insect's growth and development either by feeding inhibition or through post-ingestive phenomena (Coley *et al.*, 1985; Barbehenn *et al.,* 2001; Hoffman-Campo *et al.*, 2001; Lago *et al.,* 2002; Treutter, 2006; Jadhav *et al.,* 2012). In addition, extracts of *L. camara* and *L. cubeba* also tested positive for terpenoids. Terpenoids

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in plants can act mainly as antifeedant and growth disruptor and possess considerable toxicity toward insects (Kubo and Nakanishi, 1978; Khalid *et al.,* 1989). Saponins on the other hand are a class of phytochemicals which are reported to be insecticidal by many investigators (Marston and Hostettmann, 1985; Jeong *et al*., 2004; Sparg *et al.,* 2004; McGaw *et al.*, 2008). In the present study, saponins were found to be present in the methanol extract of *G. fragrantissima, L. cubeba and P. kesiya.* Thus, the insecticidal and antifeedant activity demonstrated by the methanol extracts of *G. fragrantissima, L. camara, L. cubeba* and *P. kesiya* could be the result of composite effect of all these classes of phytocompounds.

However, the present study is a preliminary investigation which indicates that crude methanol extracts of the four plants possess insecticidal property. Future research has to be conducted with these plants to understand their exact mode of action/s as well as isolate and identify the bioactive compound/s responsible for the toxicity demonstrated towards the target pest.

Conclusion

From the present study it can be concluded that out of seven selected plants, four plants namely, *L. camara*, *G. fragrantissima*, *L. cubeba* and *P. kesiya* have demonstrated promising insecticidal activity against *H. armigera* larvae. Further research on the bioactivity of these commonly found plants can lead to the development of a cost effective, eco-friendly formulation for crop protection, which will be beneficial to farmers of states like Meghalaya where organic farming is being encouraged by the Central and the State governments.

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اثرات لاروکشی و ضدتغذیه‌ای برخی گیاهان بومی مگالایا علیه لارو سن چهارم کرم غوزه پنبه *Helicoverpa armigera* (Hübner)

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چکیده: در این مطالعه، اثرات لاروکشی و ضدتغذیه‌ای هفت گیاه بومی شامل *Pinus kesiya* Royle (Pinaceae)، *Lantana camara* Linn. (Verbenaceae)، *Litsea cubeba* Lour. (Lauraceae)، *Gaultheria fragrantissima* Wall. (Ericaceae)، *Mikania micrantha* Kunth.(Asteraceae)، *Ambrosia artemisiifolia* Linn.(Asteraceae) و *Eupatorium riparium* Regel (Asteraceae) که در مگالایای کشور هند گسترش وسیعی دارند، علیه لاروهای سن چهارم کرم غوزه پنبه *Helicoverpa armigera* (Hübner) در شرایط آزمایشگاهی مورد بررسی قرار گرفت. تأثیر عصاره‌های تمام گیاهان با افزایش غلظت افزایش یافتند. اما با این حال تأثیر چهار گیاه شامل *L. camara*، *G. fragrantissima*، *L. cubeba*، و *P. kesiya* به‌طور معنی‌داری نسبت به کنترل منفی (حلال) و عصاره‌های *A. artemisiifolia, E. riparium* and *M. micarantha* بالاتر بود. عصاره متانولی *L. camara* موجب بالاترین مرگ‌ومیر مابین ۲۷/۷۷ و ۵۳/۳۳ درصد در غلظت‌های مورد آزمایش (۰/۲۵، ۰/۵ و ۱ درصد *w/v*) شد درحالی‌که عصاره *G. fragrantissima* بالاترین تأثیر بازدارنده تغذیه در حدود ۵۰/۹۲ و ۷۰/۶۱ درصد به‌ترتیب در غلظت‌های ۰/۱ و ۰/۵ درصد بود. عصاره برگ‌های *L. cubeba* دارای سمّیت گوارشی و ضدتغذیه‌ای زیادی در بالاترین غلظت بود. این درحالی است که عصاره برگ‌های سوزنی کاج *P. kesiya* در بالاترین غلظت اثرات سمّیت گوارشی و ضدتغذیه‌ای متوسطی داشت. تجزیه فیتوشیمی این چهار گیاه نشان داد که عصاره گیاهان فوق حاوی پنج گروه مختلف از ترکیبات شیمیایی هستند که هر کدام از آن‌ها برای آفات مضر و زیان‌بار هستند. بنابر این می‌توان نتیجه‌گیری نمود که چهار گیاه از هفت گیاه مورد مطالعه دارای تأثیر حشره‌کشی مناسبی هستند که لازم است برای تولید حشره‌کش‌های گیاهی مؤثر مورد بررسی‌های بیش‌تری قرار گیرند.

واژگان کلیدی: عصاره‌های گیاهی، سمّیت گوارشی، فعالیت ضدتغذیه‌ای، کرم غوزه پنبه، مگالایا

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