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## Efficacy of certain *Clerodendrum* leaf crude extracts against cutworm, *Spodoptera litura* Fab and cotton bollworm, *Helicoverpa armigera* Hub

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### Abstract

Efficacy of certain *Clerodendrum* leaf crude extracts were evaluated in the laboratory against third instar larvae of *Spodoptera litura* and *Helicoverpa armigera*. Toxicity was assessed through topical application method. The extracts of *C. inerme*, *C. viscosum* and *C. philippinum* were found to be more effective to both the insects. Antifeedant assay was conducted through leaf disc choice tests. Crude extracts of *C. inerme*, *C. viscosum*, *C. Phillipinum*, *C. splendens*, and *C. multiflorum* exhibited strong antifeedant activity (> 50%) at a dosage of 100-mg/ 21cm<sup>2</sup> to *S. litura*. However, the *H. armigera* showed less antifeedant activity with more feeding of extract treated leaf in all doses. The extracts of *C. inerme*, *C. viscosum* and *C. Phillipinum* were exhibiting both activities. Larval growth inhibition activity was evaluated through diet. *C. philippinum* extract was revealed strong growth inhibition to *S. litura*. Extract of *C. serratum* showed maximum (75%) growth inhibition in *H. armigera*. The other plants which were tested showed moderate effects towards these pests. Based on their efficacy, some of these plant extracts have potential for use as alternative crop protectants against lepidopteran pests.

**Keywords:** *Clerodendrum* sps, Antifeedant, *Helicoverpa armigera*, *Spodoptera litura*, Growth inhibition.

### 1. Introduction

Botanical pesticides are an important group of naturally occurring and often slow-acting crop protectants. These are generally safe to the animals and environment than the conventional insecticides with minimal residual effects [1]. Moreover, these contain mixtures of biologically active substances thus no resistance is developed in insects. Hence, the use of plant origin chemicals has been recommended and suitable alternatives for plant protection [1]. Since, botanical insecticides have been a subject of research in an effort to develop substitute to conventional insecticides [2]. The most processed forms of botanical insecticides are purified and isolated compounds from plant materials by a series of extractions and fractionations [3]. However, the preparations of plant based insecticides are initiated by screening and evaluating their biological activities in laboratory [4].

The cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae) was recognized as a major pest of tobacco only and now it has become a serious pest of tomato, cotton, castor and bitter-guard [5]. Recently, an outbreak of this pest was noticed in some districts of Tamil Nadu and Karnataka, India, on brinjal, which is not a host plant of this pest [6]. The cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae) is a polyphagous pest of worldwide occurrence causing crop damage approximately one billion dollars annually in India. This insect occurs as a major pest in many economically important crops such as pigeonpea, cotton, chickpea, blackgram and most of the vegetables [7].

Chemical nature with pharmacological and insecticidal activities of *Clerodendrum* species has been studied by several researchers [8-12]. Leaves of *C. inerme* mixed in housefly larval diet were found to reduce pupal weight and inhibit adult emergence [13]. Active component of 'Neo-clerodane' has been isolated from the leaves of *C. inerme* is responsible for growth inhibition and antifeedant activities in housefly and mosquito [8]. On the whole, *C. inerme* plant leaves has been revealed as insecticidal, antifeedant, growth inhibitor against several insect pests [11, 14-16]. Although, other *Clerodendrum* species for example, *C. calamitosum*, *C. multiflorum*, *C. paniculatum*, *C. philippinum*, *C. serratum*, *C. splendens* and *C. viscosum* have not been studied for their biological activities against insects. Therefore, the present study was

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carried out with the objectives of screening the ethanol crude extracts from eight *Clerodendrum* plants species for their insecticidal, antifeedant and growth inhibitory activities against two economically important lepidopteron insect pests *S. litura* and *H. armigera*.

## 2. Materials and Methods

### 2.1. Plant collection and extraction

The present study was carried out at Department of Zoology, Shivaji University, Kolhapur, India from July to December, 2015. The *Clerodendrum* plant species were selected for the study on the basis of availability, free from the insect attack and pungent smell. Selected plants of *C. inerme*, *C. calamitosum*, *C. multiflorum*, *C. paniculatum*, *C. philippinum*, *C. serratum*, *C. splendens* and *C. viscosum* leaves were collected in the month of July, 2015 from foothills of Western Ghats Kolhapur region. The leaves were washed with tap water to remove dust and contaminants. Leaves were shade-dried until all the moisture evaporated and pulverized by using domestic grinder. The leaf powders were subjected to Soxhlet extraction using ethanol for 16-18 hrs according to previous method [17]. The extraction processed at room temperature and then solvent was evaporated under reduced pressure in a rotary evaporator at 40 °C. Obtained crude dark-green residues were stored in a refrigerator at 4 °C for further use.

### 2.2. Insect culture

The larvae of *H. armigera* were collected from the chickpea field and *S. litura* egg masses were collected in the month of August, 2015 from the groundnut field at Kolhapur. Larvae of *H. armigera* were reared individually to avoid cannibalism on fresh cabbage leaf and *S. litura* were reared on castor leaf under laboratory conditions at 27± 2°C with 75 ± 5% relative humidity. Sterilized soil was provided for pupation. The pupae were collected from soil, sexed out and 1:1 ratio kept for adult emergence in rearing cage (50 × 50 × 50 cm). As adults emerged, 10% honey solution soaked in cotton was provided for adult. Fresh respective host plants leaves were kept in cage for oviposition and leaves were changed every day to maintain its freshness. The laboratory cultured third instars larvae of *H. armiger* as well as *S. litura* were used for all experiments.

### 2.3. Bioassays

#### 2.3.1. Insecticidal assay

The crude extract residues were dissolved in analytical grade acetone to get desired concentrations of 0.5, 1, 1.5, 2 and 2.5% and these concentrations were determined from preliminary experiments. The toxicity of extracts was determined by using topical application method as described earlier [17]. Five microliters of extract were applied on the dorsum of third instars *H. armigera* and *S. litura* larvae and control insects were received the same volume of carrier alone. In each group ten larvae and three replicates were maintained (n=30 larvae). The mortality of the larvae was recorded after 24 hrs up to 72 hrs.

#### 2.3.2. Antifeedant assay

Leaf disc choice bioassays tests carried out to determine antifeedant efficacy of *Clerodendrum* plants extracts according to Akhtar *et al.* [18]. The castor and cabbage leaf discs (21 cm<sup>2</sup> dia.) were punched using cork-borer for *S. litura* and *H.*

*armigera* respectively. Leaf discs were dipped in crude extracts of 20, 40, 60, 80 and 100 mg in acetone and control discs were sprayed carrier alone. Treated leaf discs were dried 2-3 minutes for evaporation of the solvent. A choice test was performed in a 14 cm diameter petri dish lined with moistened Whatman (No.1) filter paper. In choice test, the area was divided into equal quadrants, each quadrant containing a treated and control disc placed alternately. Three hours pre-starved third instars larvae were placed in the center of the dish. There were ten replicates for each treatment and all the treatments were repeated on 3 different days. The percentage antifeedant index was calculated according to Lewis and Van Emden [19] formula. Antifeedant index (AFI) = [(C - T) \ (C + T)] × 100 Where C is the weight of leaf disc consumed in the control and T is the weight of leaf discs consumed in the treatment.

#### 2.3.3. Growth inhibitory assay

The effect of crude leaf extracts on larval growth was assessed by oral feeding method as described by Isman [1]. Treatments were carried out as mentioned in antifeedant assay. Two pre-weighed third-instar larvae of *S. litura* (~14 mg larval wt) and *H. armigera* (~10 mg larval wt) were released in each container. The treatment was replicated 15 times and there were a total of 30 insects exposed to the treatments. After 24-hrs feeding, the larvae were transferred to the normal diet. Every day, the left-over leaves, if any and excreta of the insects were removed and provided with fresh leaves. After six days, treated as well as control larval weight was recorded for determination of growth inhibition. Per cent growth inhibition (GI) was calculated by using the El-Aswad *et al.* [20] formula. Growth inhibition (%) = [(CL-TL) / CL] × 100 Where CL is the larval weight gained in the control and TL is the larval weight gained in the treatment.

#### 2.3.4. Statistical analysis

All experimental data was corrected by Abbott's [21] formula and then statistically analyzed using ANNOVA followed by LSD at significant level of *P* < 0.05.

## 3. Results

### 3.1. Insecticidal activity

The insecticidal activities of the plant extracts were evaluated against the third instar larvae of *S. litura* and *H. armigera* by topical application method. Insecticidal activities of all plant ethanol extracts were recorded after 24 hours of exposure up to 72 hours. Toxicity results revealed that, within 24 of exposure found to be more effective than remaining two days and subsequent day's efficacy were negligible (< 8%). Therefore 24-hrs results were used for statistical analysis and presented in Table 1 and 2. Among the two insects, *H. armigera* showed more resistant with less mortality and *S. litura* showed less resistant with high mortality in all plant extracts. The maximum insecticidal activity was recorded in *C. viscosum* extract and *C. paniculatum* extract showed minimum activity in both insects Toxic efficacy of all plants extracts were dose as well as duration dependent. Nevertheless, all plant leaves extracts demonstrated less insecticidal activities when compare to *C. inerme* in both insects, though the *C. viscosum* results were on par with *C. inerme*.

**Table 1:** Insecticidal efficacy of *Clerodendrum* species extracts against 3<sup>rd</sup> instar larvae of *S. litura* by topical application

Conc. (%)	Percent mortality of <i>S. litura</i> *							
	<i>C. inerme</i>	<i>C. viscosum</i>	<i>C. philippinum</i>	<i>C. splendens</i>	<i>C. multiflorum</i>	<i>C. calamitosum</i>	<i>C serratum</i>	<i>C. paniculatum</i>
0.5	29.10±1.22e	17.17±1.11e	7.77±1.11e	12.20±1.11e	11.11±1.11e	11.11±1.11d	7.77±1.11d	7.77±1.11c
1	48.88±1.11d	28.88±1.11d	24.88±1.11d	17.77±1.11d	25.55±1.11d	14.40±1.11d	14.44±1.11c	12.22±1.11b
1.5	64.43±1.11c	48.88±1.11c	41.10±1.10c	37.77±1.11c	28.88±1.11c	27.70±1.11c	27.77±1.11b	24.43±1.11a
2	70.00±0.03b	57.77±1.11b	55.54±1.11b	48.88±1.11b	44.43±1.11b	34.44±1.11b	29.99±0.003ab	26.66±1.11a
2.5	76.66±1.92a	73.33±1.92a	65.55±1.11a	61.10±1.11a	47.77±1.11a	48.88±1.11a	33.33±11.92a	27.77±11.92a

\* Mean of three replications. Means followed by the same letter in column are not significantly different (ANNOVA followed by LSD at  $P < 0.05$ )

**Table 2:** Insecticidal efficacy of *Clerodendrum* species extracts against 3<sup>rd</sup> instar larvae of *H. armigera* by topical application

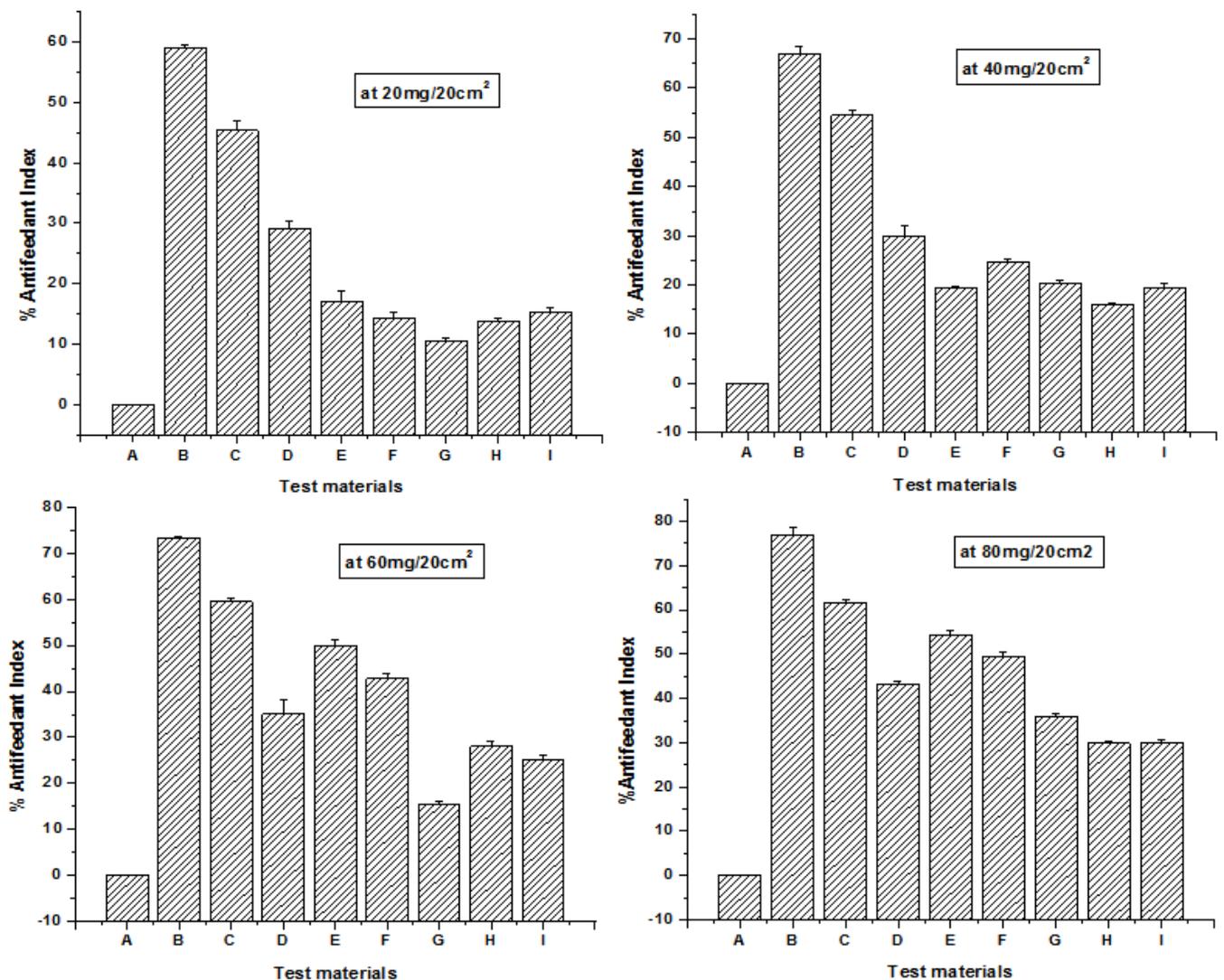
Conc. (%)	Percent mortality of <i>H. armigera</i> *							
	<i>C. inerme</i>	<i>C. viscosum</i>	<i>C. philippinum</i>	<i>C. splendens</i>	<i>C. multiflorum</i>	<i>C. calamitosum</i>	<i>C serratum</i>	<i>C. paniculatum</i>
0.5	6.66±0.0e	5.55±1.11e	4.44±1.11e	4.44±1.11d	3.33±1.11e	4.44±0.0e	3.33±0.0e	3.33±0.0e
1	14.44±2.22d	8.88±1.11d	6.66±0.0d	7.77±1.11d	7.77±1.11d	12.22±1.11d	6.66±0.0d	4.43±1.11d
1.5	26.66±1.92c	15.55±1.11c	8.88±2.93c	15.55±1.11c	15.55±1.11c	21.11±1.11c	8.88±1.11c	7.77±1.11c
2	38.88±1.11b	32.22±1.11b	23.32±1.92b	22.22±1.11b	21.11±1.11b	25.55±1.11b	2.22±1.11b	12.22±1.11b
2.5	58.88±1.11a	53.32±1.92a	49.99±0.003a	38.88±1.11a	25.55±1.11a	32.22±1.11a	19.99±0.003a	15.55±1.11a

\* Mean of three replications. Means followed by the same letter in column are not significantly different (ANNOVA followed by LSD at  $P < 0.05$ )

### 3.2. Antifeedant activity

The extracts showed a significant deterrence of food consumption at different doses ( $P < 0.05$ ). Among the plant extract evaluated at 100 mg concentration, *C. inerme*, *C. viscosum*, *C. Phillipinum*, *C. splendens*, and *C. multiflorum* showed significantly ( $P < 0.05$ ) highest antifeedant potential with AFI ( $> 50\%$ ) followed by *C. calamitosum* and *C. serratum* ( $< 50\%$ ) to *S. litura*. The lowest antifeedant activity was showed by the extract of *C. panniculatum* plant (Fig. 1).

As compare to *S. litura*, the *H. armigera* showed less antifeedant activity with more feeding of extract treated leaf in all doses (Fig. 2). On the other hand, the antifeedant index of extracts demonstrated that, at 100 mg concentration, the *C. inerme*, *C. viscosum*, *C. multiflorum* and *C. splendens* plant extracts revealed highest antifeedant activity ( $< 40\%$ ). The plant extracts of *C. calamitosum* and *C. paniculatum* demonstrated moderate antifeedant activity and *C. philippinum* and *C serratum* showed lowest activities.



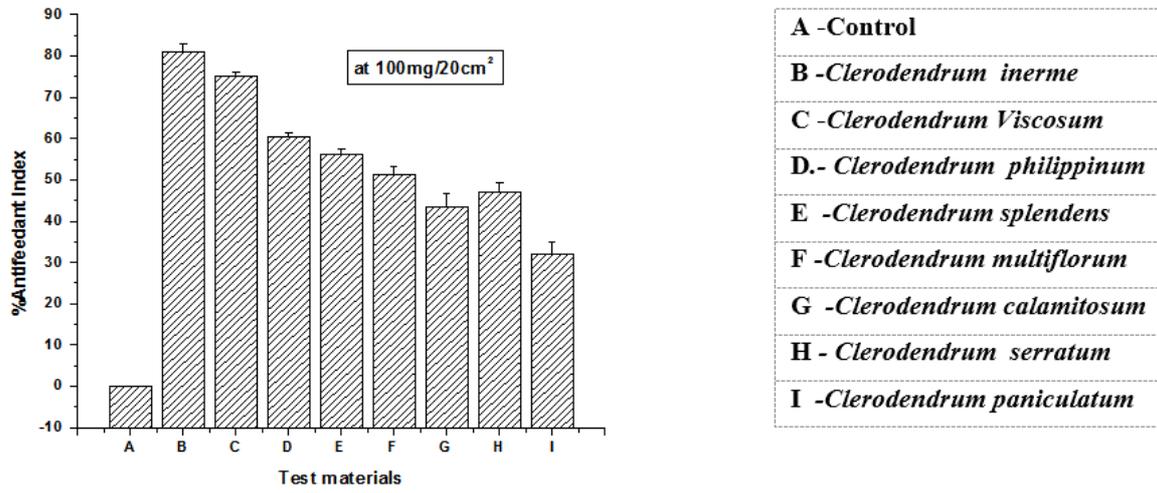


Fig 1: Antifeedant efficacy of *Clerodendrum* species extracts against third instar *S. litura* larvae after 24-hrs application

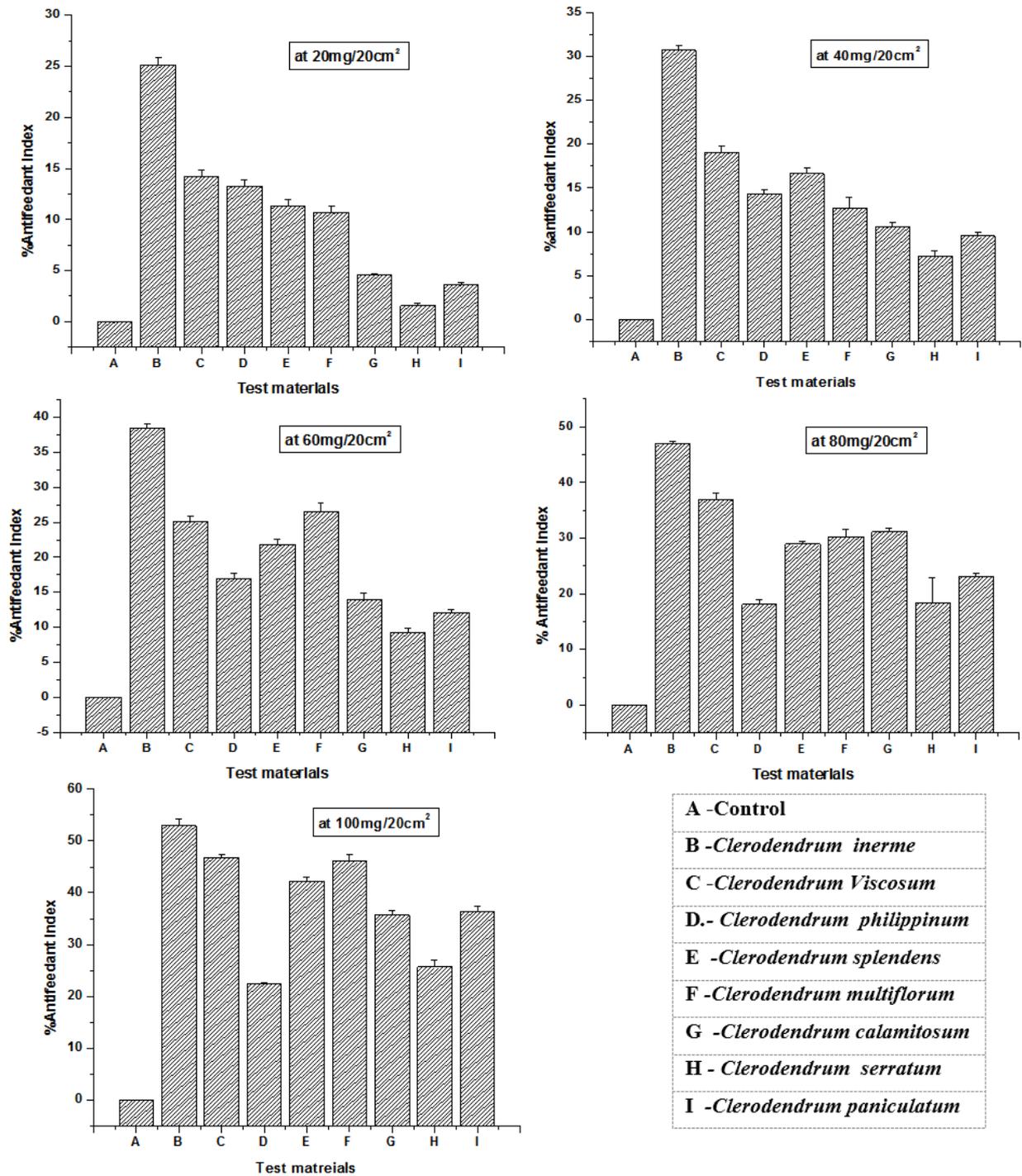


Fig 2: Antifeedant efficacy of *Clerodendrum* species extracts against third instar *H. armigera* larvae after 24-hrs application

### 3.3. Growth inhibition activity

All plant ethanol extracts were demonstrated larval growth inhibition in dose-dependent manner after six days of feeding. The leaf extract of *C. philippinum* was found to be most potent with 66% growth inhibition in *S. litura* among all extracts tested (Table 3). On the contrary, the crude extract of *C.*

*splendens* demonstrated less (57%) growth inhibition when compare to other extracts. The crude extract of *C. serratum* showed maximum 75% larval growth inhibition in *H. armigera* (Table 4). All plants tested on both insect, *S. litura* larval growth inhibitions results showed more than the *H. armigera*.

**Table 3:** Growth inhibition efficacy of *Clerodendrum* species extracts against 3<sup>rd</sup> instar larvae of *S. litura*

Extract Conc. (%)	Percent growth inhibition*							
	<i>C. inerme</i>	<i>C. viscosum</i>	<i>C. philippinum</i>	<i>C. splendens</i>	<i>C. multiflorum</i>	<i>C. calamitosum</i>	<i>C. serratum</i>	<i>C. paniculatum</i>
0.5	18.87 ±1.09 <sup>c</sup>	24.94 ±1.89 <sup>d</sup>	31.54 ±2.66 <sup>d</sup>	22.18 ±3.27 <sup>d</sup>	24.77 ±2.15 <sup>d</sup>	19.45 ±2.80 <sup>d</sup>	28.46 ±1.66 <sup>d</sup>	27.60 ±2.72 <sup>e</sup>
1	26.65 ±4.51 <sup>c</sup>	36.15 ±1.99 <sup>c</sup>	39.06 ±1.90 <sup>e</sup>	25.90 ±3.01 <sup>cd</sup>	30.69 ±2.50 <sup>d</sup>	25.01 ±2.82 <sup>d</sup>	33.38 ±4.43 <sup>cd</sup>	36.11 ±1.39 <sup>d</sup>
1.5	41.95 ±4.81 <sup>b</sup>	45.60 ±2.19 <sup>b</sup>	47.32 ±2.96 <sup>b</sup>	32.02 ±3.36 <sup>c</sup>	38.41 ±2.12 <sup>c</sup>	37.05 ±1.96 <sup>c</sup>	42.33 ±3.77 <sup>bc</sup>	44.52 ±1.51 <sup>c</sup>
2	54.61 ±4.19 <sup>a</sup>	51.64 ±1.84 <sup>b</sup>	62.39 ±2.25 <sup>a</sup>	48.53 ±2.13 <sup>b</sup>	47.79 ±2.87 <sup>b</sup>	47.21 ±1.38 <sup>b</sup>	49.64 ±3.82 <sup>b</sup>	52.09 ±1.49 <sup>b</sup>
2.5	60.91 ±4.11 <sup>a</sup>	59.43 ±1.76 <sup>a</sup>	65.77 ±1.33 <sup>a</sup>	57.06 ±2.15 <sup>a</sup>	61.49 ±1.80 <sup>a</sup>	61.11 ±1.40 <sup>a</sup>	62.60 ±2.84 <sup>a</sup>	61.34 ±0.72 <sup>a</sup>

\* Mean of three replications. Means followed by the same letter in column are not significantly different (ANNOVA followed by LSD at  $P < 0.05$ )

**Table 4:** Growth inhibition efficacy of *Clerodendrum* species extracts against 3<sup>rd</sup> instar larvae of *H. armigera*

Conc. (%)	Percent growth inhibition*							
	<i>C. inerme</i>	<i>C. viscosum</i>	<i>C. philippinum</i>	<i>C. splendens</i>	<i>C. multiflorum</i>	<i>C. calamitosum</i>	<i>C. serratum</i>	<i>C. paniculatum</i>
0.5	12.39 ± 1.08 <sup>d</sup>	16.63 ± 0.35 <sup>e</sup>	12.86 ± 0.61 <sup>d</sup>	24.47 ± 1.96 <sup>d</sup>	20.48 ± 0.57 <sup>e</sup>	14.39 ± 0.67 <sup>d</sup>	20.98 ± 1.27 <sup>e</sup>	19.58 ± 3.25 <sup>e</sup>
1	37.59 ± 1.24 <sup>c</sup>	34.53 ± 1.71 <sup>d</sup>	46.46 ± 0.96 <sup>e</sup>	39.64 ± 1.26 <sup>e</sup>	30.78 ± 1.40 <sup>d</sup>	45.04 ± 2.53 <sup>c</sup>	26.78 ± 0.89 <sup>d</sup>	36.92 ± 0.92 <sup>d</sup>
1.5	38.41 ± 1.37 <sup>c</sup>	47.64 ± 0.49 <sup>c</sup>	49.01 ± 1.73 <sup>c</sup>	56.71 ± 3.64 <sup>b</sup>	55.09 ± 1.21 <sup>c</sup>	49.63 ± 4.03 <sup>c</sup>	46.56 ± 1.92 <sup>c</sup>	51.39 ± 0.69 <sup>c</sup>
2	58.32 ± 1.22 <sup>b</sup>	53.50 ± 0.91 <sup>b</sup>	55.86 ± 1.64 <sup>b</sup>	62.11 ± 2.08 <sup>b</sup>	61.59 ± 1.60 <sup>b</sup>	57.72 ± 1.52 <sup>b</sup>	63.50 ± 0.70 <sup>b</sup>	58.28 ± 1.00 <sup>b</sup>
2.5	69.48 ± 1.58 <sup>a</sup>	65.39 ± 0.77 <sup>a</sup>	68.27 ± 1.40 <sup>a</sup>	71.82 ± 0.49 <sup>a</sup>	68.25 ± 1.44 <sup>a</sup>	67.02 ± 0.36 <sup>a</sup>	75.50 ± 1.23 <sup>a</sup>	71.68 ± 0.43 <sup>a</sup>

\* Mean of three replications. Means followed by the same letter in column are not significantly different (ANNOVA followed by LSD at  $P < 0.05$ )

### 4. Discussion

Generally, plant extracts contain a number of biologically active substances and their qualitative and quantitative composition closely depends on the applied extraction techniques. The highest extraction yield from aerial parts of plant was obtained by Soxhlet extraction [17] with ethanol as a solvent [12] hence, in the present study ethanol was used for extraction. It has been proved that ethanol is a suitable solvent for terpenoids extraction from plant materials [22] and terpenoids are the main biological active components in *Clerodendrum* species [10]. Our attention was focused on extracts the terpenoids, which are generally considered as contact and respiratory toxins [23] and in addition they are responsible for short-term mortality in insects [24].

Insecticidal results revealed the effects of plant extracts on major agricultural pests of *S. litura* and *H. armigera*. Significant insecticidal and antifeedant activities against both insect larvae were observed in crude extracts of *C. inerme*, *C. viscosum* and *C. multiflorum* than other plants tested. Isman [23] and Pavela [24] reported that majority of terpenoid compounds showed contact toxicity activity. Ethanol extracts of the seeds of *T. priureana*, *T. roka* and *T. connaroides* showed high levels of insecticidal and antifeedant activities in leaf disc method against *S. frugiperda* [25]. Devanand and Usha Rani [26] results demonstrated that, high toxic effects of acetone extracts of *T. grandis*, *M. indica* and *M. charantia* to *S. litura* and *A. janata* and additionally the extracts of *M. charantia*, *T. grandis*, *M. indica* and *T. indica* exhibited strong antifeedant activity (> 85%) in leaf disc bioassays at a dosage of 100-mg/21cm<sup>2</sup> against *S. litura* and *A. janata*. The crude seed ethanol

extracts of *Annona squamosa* having toxic and antifeedant potential against lepidopteran pests of *P. xylostella* and *T. ni* [27].

The food consumption rate of the *S. litura* and *H. armigera* was affected by the plant extracts with the increasing concentrations of *C. viscosum* and *C. philippinum* than other extracts. Antifeedant results indicate that both the insect species showed a significant increase in feeding deterrent response. Several investigators have been reported that phytochemicals offer antifeedant activity against *S. litura* and *H. armigera* [28, 29]. The results of Valsala and Gokuldas [30] reported that the petroleum ether crude extract of *C. infortunatum* showed repellent and oviposition deterrence against *C. chinensis* For instance, Pavunraj *et al.* [31] stated that leaf crude from *Pergularia daemia* exhibited good antifeedant activity against *H. armigera* and *S. litura*. Root ethanol extract of *P. murex* exhibited good antifeedant activity against *S. litura* [4]. The extract of *Adhatoda vasica* leaves was found to have feeding deterrent properties when applied on leaf discs method to *S. littoralis* [32]. Similarly, Devanand and Usha Rani [26] reported that acetone extracts of 15 plant leaves showed excellent antifeedant and toxic properties against *S. litura*.

In addition to toxic and antifeedant effects, these *Clerodendrum* plant extracts exhibited growth inhibitory activity against both the test larvae. Our results clearly indicates that, ethanol extracts of *C. philippinum*, *C. calamitosum* and *C. serratum* were potent growth inhibitors to *S. litura* and *H. armigera* among the plant extracts tested. Leaves extract of *C. inerme* mixed in housefly larval diet were found to reduce puparial weights and inhibit adult emergence [13]. The growth inhibition activities of the extracts of several

Meliaceae plants such as *Azadirachta indica* [33], *Melia azedarach* [34], *Melia toosendan* [35] and *Aglaia* species [36] have been extensively evaluated on several insect pests. Ethyl acetate extract from *Syzygium lineare* [37], methanol extract of *Melia dubia* [38] showed growth inhibitory activity against *S. litura*. Janprasert *et al.* [39] reported the isolated fractions compounds from *A. odorata* have feeding inhibition and growth regulating activity against *S. littoralis*.

## 5. Conclusion

The biological activities of these extracts suggest a future exploitation of the materials in to potential insect management alternatives with a minimum environmental impact. It is beneficial, as the extracts of *C. inerme*, *C. viscosum* and *C. philippinum* at higher doses act as toxicant, while the lower dilution of the same plant is antifeedant. The results implying the dual function of a single plant material in these lepidopteran pests management by chosen plant extracts. It also suggests that by a single application of these compounds a complete success of the insect control can be achieved.

## 6. Acknowledgement

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