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## Pesticidal properties of *Anamirta cocculus*, *Cardiospermum halicacabum*, *Cocculus laurifolius* and *Strychnos nux-vomica* against *Spodoptera litura* (Lepidoptera: Noctuidae)

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*Spodoptera litura*, commonly known as taro caterpillar, is a major pest of several vegetables and economically important crops. The pest is reported to reduce the yield of the affected crop up to 10-30%. Hence, controlling this pest is one of the very crucial steps in increasing crop yield. The extensive use of broad-spectrum chemical pesticides to control *S. litura* has many negative impacts. The present study is an attempt to evaluate the efficacy of certain plants against *S. litura*. In this study, we investigated the anti-insect properties of leaf and bark extracts of *Anamirta cocculus*, *Cardiospermum halicacabum*, *Cocculus laurifolius* and *Strychnos nux-vomica*. All four plant extracts showed significant anti-feedant activity compared to control at different concentrations. The leaf extracts showed the activity in the order *S. nux-vomica* > *C. halicacabum* > *C. laurifolius* > *A. cocculus* at the exposure of maximum concentration. Anti-feedant activity of the bark extract was in the order *C. laurifolius* > *S. nux-vomica* > *C. halicacabum*. The leaf extracts of three plants *A. cocculus*, *C. halicacabum* and *S. nux-vomica* showed significant repellent activity. The repellent activity of the bark extracts was in the order, *C. laurifolius* > *C. halicacabum* > *S. nux-vomica* > *A. cocculus*. The GC-MS analysis of these plant extracts have shown many compounds with known anti-insect properties and specific molecule-based bio-assays might be required to ascertain the distinctive effects of these compounds.

**Keywords:** *Anamirta cocculus*, *Cardiospermum halicacabum*, *Cocculus laurifolius*, *Strychnos nux-vomica*, *Spodoptera litura*, Anti-insect activities.

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### Introduction

*Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) is a poly-phytophagous insect damaging several vegetables and field crops in many Asian countries including India. It is commonly known as cotton cutworm, rice cutworm, taro caterpillar, tobacco budworm, cotton leafworm, cluster caterpillar, cotton worm, Egyptian cotton leafworm, tobacco caterpillar, tobacco cutworm, tobacco leaf caterpillar and common cutworm. *S. litura* has been reported from the south and south-east Asia, Australia and pacific islands<sup>1</sup>. This insect deteriorates leaves of many commercially important crops. Earlier it was identified as a random pest of tobacco, but over the years it has become a major pest in tobacco<sup>2-4</sup> as well as

many other plants. Among the 44 families of plants, there are 150 cultivated food plants in the world<sup>5</sup> and 60 from India that come under the attack of this pest<sup>6</sup>. Some plants under its infestation include castor, cotton, sunflower, cabbage, pigeon pea, chili, cucumber, pumpkin, potato, banana, tomato, okra, etc<sup>7,8</sup>. This pest has caused yield reduction up to an extent of 30% in crops<sup>9-15</sup>. An average of 15% of crops worldwide is currently damaged by insects, so controlling pests is crucial in achieving the goal of increasing crop yield<sup>16</sup>.

*S. litura* is one of the most damaging pest, which consumes up to 85.5% of the leaf area<sup>17</sup>. The assessment of an economic impact of this polyphagous pest in a range of crops and trees were studied well in India and abroad<sup>18-21</sup>. Recently, there were many incidences reported on the reduced qualitative and quantitative yield in soybean and cotton from Maharashtra. The larvae adversely affect flowers, flower buds and bolls by eating their contents. It was estimated that about 3-

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4 buds per each day can be damaged by a single larva and 7-8 larvae can destroy one adult plant in a day<sup>22</sup>.

Due to the extensive use of broad-spectrum pesticides to control *S. litura*, it has developed resistance to many of these chemical agents. Concurrently these chemical pesticides are harmful to the non-target organisms as well as humans<sup>23</sup>. The widespread usage of broad-spectrum synthetic pesticides during the last century has caused numerous environmental problems such as pest resistance, increased cost of agricultural production, retention of pesticide residues and non-target toxicity. Thus, there is a need for developing ecologically safe bio-agents, which could be used against these pests. In such a situation, new agents originating from plant products could be helpful in more environment-friendly integrated pest management and function as an effective alternative to chemical pesticides.

Botanical pesticides are often slow-acting and safer to non-target organisms and human health. Role of secondary metabolites in insect-plant interaction is under constant exploration. Plants produce a variety of natural products with highly diverse chemical natures and that protect them from pest attack<sup>23</sup>. These chemical substances function in many ways, such as repellents, anti-feedants, etc. Anti-feedants inhibit the feeding of insect on a treated food material without killing or repelling<sup>24</sup>. Repellents deter an insect from flying to, landing on or biting human or skin of an animal or eating a food material<sup>25</sup>. Many plants have these kinds of molecules and evaluating their properties may provide newer molecules with promising anti-insect properties.

This study was framed to evaluate the efficacy of *A. cocculus*, *C. halicacabum*, *C. laurifolius*, and *S. nux-vomica* plant extracts against *S. litura*. Different pesticidal effects such as anti-feedant activity, repellent activity, and contact toxicity against *S. litura* were evaluated along with the chemical composition of the extracts.

## Materials and Methods

### Plant materials and extraction

The leaves and bark of the plants, *A. cocculus*, *C. halicacabum*, *C. laurifolius*, and *S. nux-vomica* were collected from foothills of southern Western Ghats. Plant specimens were identified by plant taxonomist Dr P. Sujanapal, Kerala Forest Research Institute, Peechi, Kerala, India. Voucher specimens (*A. cocculus* -18024, *C. halicacabum* - 18025, *C. laurifolius* - 18026 and *S. nux-vomica* - 18027)

were deposited in Kerala Forest Research Institute Herbarium (KFRI), Kerala, India.

The collected plant materials were thoroughly washed, shade dried and powdered with the help of a blender. The plant powder (10 g) was extracted with 200 mL methanol, in a flask of 500 mL capacity, using soxhlet apparatus. Four to five repeat refluxes were carried out for each plant sample (total time of 6–8 h). After extraction, the methanol extract was concentrated to near dryness under reduced pressure maintaining the temperature below 40 °C using a rotary evaporator. The samples were stored at deep freezer (-20 °C) until further use. The aqueous methanolic extract was used for the study. This was prepared by dissolving the dried extract powder in 0.01% of methanol and made up to the final volume with water. Methanol (0.01 %) in water was used as control.

### Rearing of insects

Different stages of *S. litura* were collected from banana fields of Ernakulam (Kerala), India and their subsequent generations were maintained at 25±1 °C, 60±5% relative humidity. Plastic containers covered with muslin cloth were used for insect culture in the laboratory, and the larvae were reared on *Ricinus communis* (castor) leaves which were changed daily. During the pupation stage, it was shifted to jars containing moist sterilized sand covered with filter paper. Just after the adult emergence, they were transferred to oviposition jars and provide the honey solution with few drops of multivitamin to increase the rate of fecundity as food, which was soaked in cotton attached on the sides of the jars. To facilitate the egg-laying, the oviposition jars were lined with filter paper. Neonates, upon hatching from the eggs, were transferred to glass jars containing fresh thoroughly washed *R. communis* (castor) leaves. This process was repeated and the insect culture was maintained throughout the study period.

### Bio-assay of plant extracts against *S. litura*

#### Anti-feedant and feeding activity

Anti-feedant activity of plant extracts was studied using leaf disc no-choice bioassay method<sup>26</sup>. Fresh castor leaf discs (4.5 cm diameter) were dipped in 0.5, 1.0, 2.5, and 5.0% concentrations of crude aqueous methanolic extracts against *S. litura* in individual boxes. The leaf disc treated with methanol in water was used as the control. In each box, wet cotton was placed to avoid early drying of the leaf discs and

single third instar larva was introduced. Since the maximum feeding activity and leaf damage was observed in the third instar stage of the larvae, the assay was carried out using the third instar of the larvae. Progressive consumption of treated or control leaf area by the larvae after 24 hours was recorded using graph paper. Leaf area, eaten by larvae in treatment was corrected from the control and shrinkage percentage. Four replications were maintained for each treatment. The per cent of feeding and anti-feedant activity in the no-choice method was calculated based on the following formula<sup>27</sup>.

$$\text{Per cent feeding} = \frac{(\text{Initial leaf disc area for feeding} - \text{area left over after feeding})}{\text{Initial leaf disc area given for feeding}} \times 100$$

$$\text{Antifeedant activity(\%)} = \frac{(\% \text{ protection in treatment} - \% \text{ protection in control})}{(100 - \% \text{ protection in control})} \times 100$$

#### Repellent activity

The repellent of the insect was tested with choice bioassay<sup>28</sup>. The fresh castor leaf disc of 4.5 cm diameter was treated with plant extracts (100 mg/mL) on one piece and with control on another. Then the leaves were exposed to ten, 3<sup>rd</sup> instar larvae of *S. litura* by placing them in the middle of each box. After three hours, the number of larvae present at treated or control was counted. Repellent index (RI) was calculated as

$$\text{Repellent Index} = \frac{(C - T)}{(C + T)} \times 100$$

Where, C = Number of larvae in the control diet and T = Number of larvae in the treated diet. If RI >50, the extract is repellent and RI <50, the extract is non-repellent<sup>29</sup>. All the experiments were repeated four times.

#### Contact toxicity

This experiment tested the hypothesis that topically applied plant extract solutions exhibit contact toxicity to *S. litura* larvae. Contact toxicity of the plant extracts was evaluated using 1<sup>st</sup> instar larvae as the contact toxins show maximum effect in the younger stage. For each replicate, 10 larvae were transferred to a Whatman No. 1 filter paper disc in a 90 mm disposable plastic box. Three replicates of 10 larvae each were treated with each plant extract. Each larva was treated topically with aqueous-methanol plant extract using a 50  $\mu$ L micropipette. In the control treatment, larvae were treated with methanol in water. After treatments, it was allowed to dry for 10 minutes

at 25 $\pm$ 1  $^{\circ}$ C and were subsequently transferred individually into castor leaves containing plastic containers. Following treatment application, larvae were maintained at 25 $\pm$ 1  $^{\circ}$ C and mortality was assessed after 24 hours. The experiment was repeated four times.

#### Gas chromatography-mass spectrometry analysis

The chemical composition of the plant extracts in methanol was analyzed using GC-MS. The extracts were filtered through 0.22  $\mu$ m syringe filter before the analysis. One microlitre of the filtered sample was analyzed using GC-MS (QP-2010-S Shimadzu) equipped with Rxi-5Sil MS column of 30 m in length, 0.25 mm in diameter, and 0.25  $\mu$ m thickness. The GC-MS was employed with helium as the carrier gas at a constant flow of 1 mL/min. The oven temperature started at 80  $^{\circ}$ C and remained at this temperature for 4 minutes increasing to 280  $^{\circ}$ C at 5 $^{\circ}$  C/min ramp rate. Injection port was adjusted at 260  $^{\circ}$ C and splitless injection mode was used. EI mode was at 70 eV, while a mass spectrum was recorded in the 50–500 amu range and ion source temperature was maintained at 200  $^{\circ}$ C. The components of the extracts were identified by comparing the retention times of chromatographic peaks using quadrupole detector with NIST and Wiley library.

#### Statistical analysis

Data were expressed as mean $\pm$ SE from four replicates per each treatment. Data of anti-feedant and feeding activity were analyzed by one-way analysis of variance followed by Dunnett's test for comparison between respective control and treatment groups. For the experiments of contact toxicity and repellent activity, data were analyzed by one-way analysis of variance followed by Student-Newman-Keul's multiple mean comparison test. The level of significance was set at  $p \leq 0.05$ . Data of all the results in this study were obtained from at least three independent experiments with similar pattern.

## Results and Discussion

#### Bio-assay of plant extracts against *S. litura*

##### Anti-feedant and feeding activity

The results of the anti-feedant activity of the leaf extracts were shown in Fig. 1a. The leaf extracts showed significant anti-feedant activity at higher concentrations, i.e., 2.5 and 5%. Among the concentrations of extracts tested, leaf extracts of *S. nux-vomica* showed the maximum anti-feedant

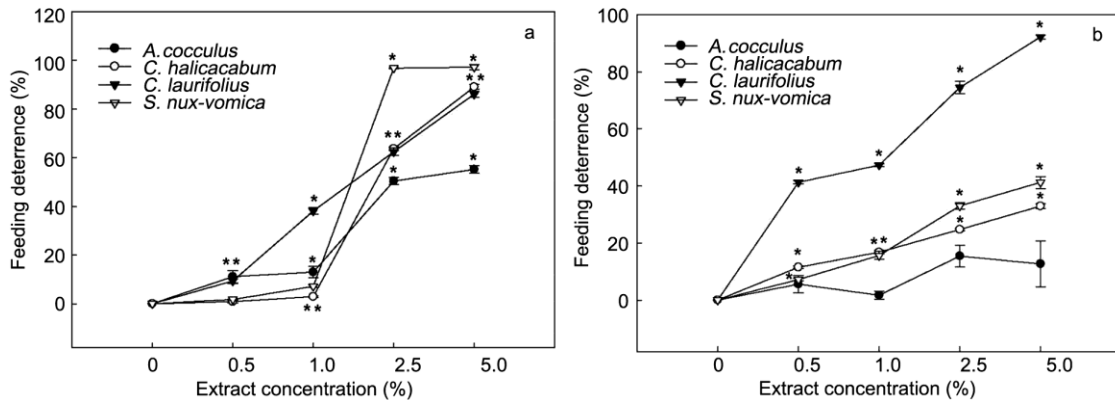


Fig. 1 — Anti-feedant activity of leaf (a) and bark (b) methanol extracts of *A. cocculus*, *C. halicacabum*, *C. laurifolius* and *S. nux-vomica* against *Spodoptera litura*. The values are expressed as mean  $\pm$  SE of four replicates per experiment. \* Significantly different from respective controls at  $p \leq 0.05$  by Dunnet's test.

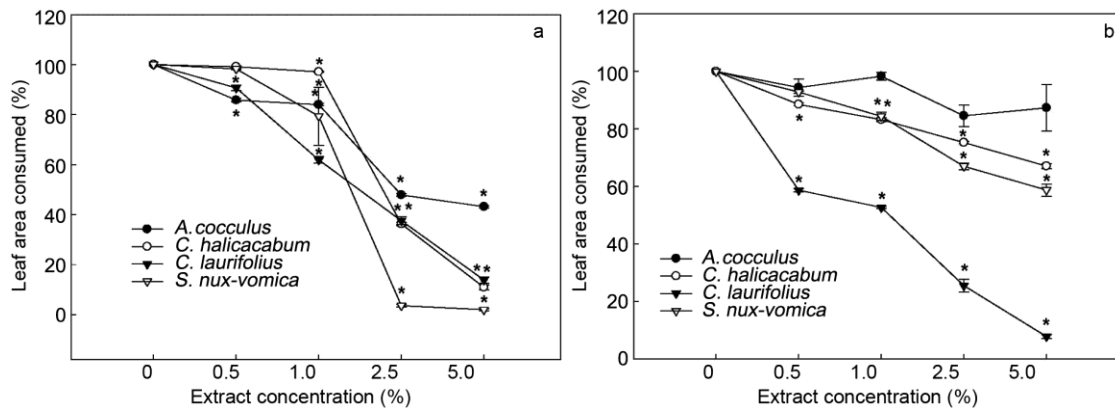


Fig. 2 — Feeding activity of leaf (a) and bark (b) methanol extracts of *A. cocculus*, *C. halicacabum*, *C. laurifolius* and *S. nux-vomica* against *Spodoptera litura*. The values are expressed as mean  $\pm$  SE of four replicates per experiment. \* Significantly different from respective controls at  $p \leq 0.05$  by Dunnet's test.

activity at an exposure of 2.5% (96.8%) and 5% (97%) extracts. *C. laurifolius* extracts showed significant anti-feedant activity from 1% exposure onwards and maximum activity was observed at 5% exposure (86.11%). In *C. halicacabum* significant anti-feedant activity was observed from 1% exposure and the maximum anti-feedant activity was noted in 5% extract exposure (89.06%). A similar trend was noted in *A. cocculus* with maximum anti-feedant activity (55.2%) was seen in the highest dose of exposure.

However, a different pattern was noted in the assay of bark extracts. The maximum activity was noted in *C. laurifolius* extracts with 41.29% anti-feedant activity on the exposure of 0.5% extract followed by 47.29% in 1% exposure, 74.46% in 2.5% exposure and a maximum of 92.17% in 5% exposure. *C. halicacabum* and *S. nux-vomica* showed a similar trend with a maximum anti-feedant activity of 32.93%

and 41.23% respectively at the exposure of 5% bark extracts. Even though *A. cocculus* extracts showed significant anti-feedant activity compared to control, it has the lowest anti-feedant activity (12.66%) even at an exposure of 5% extract. The results are shown in Fig. 1b. Similar to anti-feedant activity assay, feeding activity was also recorded on the exposure of leaf and bark extracts of *A. cocculus*, *C. halicacabum*, *C. laurifolius* and *S. nux-vomica*. In all the samples, 100 % feeding was recorded in untreated control samples. In leaf extracts (Fig. 2a), least feeding activity (1.98%) was noted in *S. nux-vomica* extracts at 5% extract exposure, followed by *C. halicacabum* (10.92%), *C. laurifolius* (13.87%) and *A. cocculus* (43.2%). In bark extracts, *C. laurifolius* has shown the maximum inhibition in all the concentrations, 58.69 % in 0.5%, 52.70% in 1%, 25.52% in 2.5% and 7.8% in 5% extracts. This was followed by *S. nux-vomica*, *C. halicacabum* and *A. cocculus* (Fig. 2b).

In the present study, *S. nux-vomica* leaf extract had shown up to 97% anti-feedant activity which is highest compared to all other extracts. *C. halicacabum* and *C. laurifolius* also showed a similar feeding inhibition at highest dose of exposure. In case of bark extracts, *C. laurifolius* had the maximum effect followed by *S. nux-vomica* and *C. halicacabum*. Anti-feedant activity of leaf extracts of *Catharanthus roseus* and *Ocimum sanctum* against the 4<sup>th</sup> instar larvae of *S. litura* had shown promising results<sup>18</sup>. On screening of different plant extracts against *S. litura*, varying degrees of anti-feedant activity was reported i.e., *Pedaliium murex* (87.49%), *Lantana camara* (83.17%), *Gymnema sylestre* (63.08%), *Taxodium disticum* (56.24%) and *Ageratum vulgare* (51.27%)<sup>30</sup>. Anti-feedant and growth inhibitory activities of *Syzygium lineare*<sup>31</sup> and flower extract of *Cassia fistula*<sup>32</sup> has been reported against *S. litura*. The crude acetone extracts of *Tamarindus indica*, *Tectona grandis*, *Madhuca indica*, *Jatropha curcas* and *Momordica charantia* showed significant anti-feedant activity against *S. litura*<sup>33</sup>. The results of the present study were well corroborated with many of the above-cited results. The extracts of *S. nux-vomica* and *C. laurifolius* showed better anti-feedant activity compared to many other plants reported earlier, this could be because of the presence of molecules with anti-feedant activities in them. Most potent insect anti-feedants are indole alkaloids, quinoline, diterpenoids, triterpenoids and sesquiterpene lactone molecules present in the plants<sup>34</sup>.

#### Repellent activity

Repellent index (RI) of both leaf and bark extracts (100 mg/mL) of *A. cocculus*, *C. halicacabum*, *C. laurifolius* and *S. nux-vomica* were tested

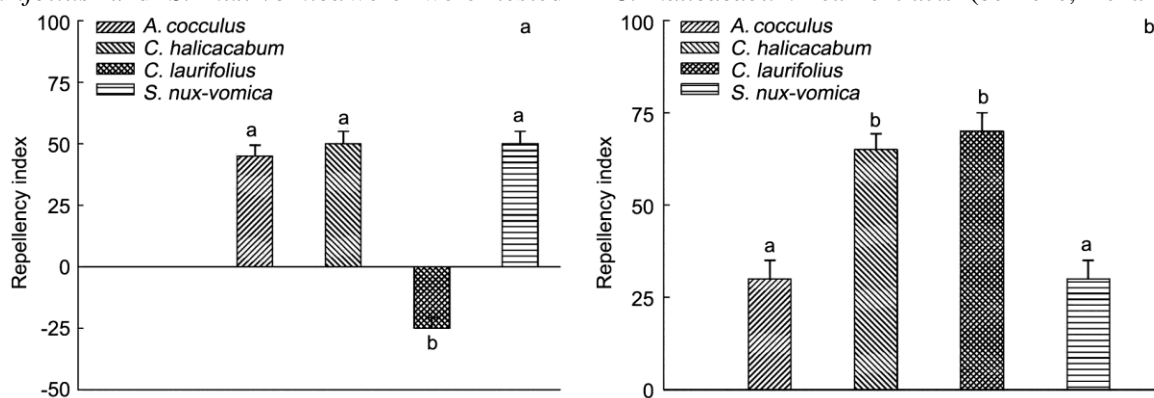


Fig. 3 — Repellent activity of leaf (a) and bark (b) methanol extracts of *A. cocculus*, *C. halicacabum*, *C. laurifolius* and *S. nux-vomica* against *Spodoptera litura*. The values are expressed as mean  $\pm$  SE of four replicates per experiment. Means followed by different alphabets are significantly different at  $p \leq 0.05$  by Student-Newman-Keul's multiple comparison test.

in the 3<sup>rd</sup> instar larvae of *S. litura*. In leaf extracts (Fig. 3a), RI was almost similar in *A. cocculus* (45), *C. halicacabum* (50) and *S. nux-vomica* (50). No statistically significant difference was noted among these plants. However, the *C. laurifolius* had shown an attractant (-25) activity. In bark extracts (Fig. 3b), *A. cocculus* (30) and *S. nux-vomica* (30) were similar in their RI. Similarly, *C. halicacabum* (65) and *C. laurifolius* (70) falls into the same group. Among the extracts tested, bark extracts of *C. laurifolius* has shown the maximum RI. The study revealed that the crude plant extracts showed different levels of repellent activity against *S. litura*. *A. cocculus*, *S. nux-vomica* and *C. halicacabum* leaf extracts showed RI values not significantly different from each other. However, bark extracts *C. halicacabum* and *C. laurifolius*, have shown significantly higher values compared to other two plants. Repellent activity of *Caulerpa scalpelliformis* extracts and its formulations against *S. litura* has been reported earlier<sup>35</sup>. Arthropods show a differential response to volatile plants. At the same time, the same compound is attractive to some arthropods and repellent to others. The volatile compounds in orange fruit *Citrus aurantium* (L.) were attractive to *Anastrepha ludens* and repellent for *Culex pipiens*<sup>36,37</sup>. *C. halicacabum* crude extract showed protection against mosquito bites without any allergic reaction to the test person, and also, the repellent activity was dependent on the strength of the plant extracts. The tested plant extracts had exerted promising repellent activity against three mosquitoes *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi*<sup>38</sup>. Adulticidal properties of *C. halicacabum* plant extract against these three important vector mosquitoes have also been reported<sup>39</sup>. *C. halicacabum* leaf extracts (benzene, hexane, ethyl

acetate, methanol, and chloroform) were found to have larvicidal and ovicidal activity against *C. quinquefasciatus* and *A. aegypti*. The ovicidal activity was maximum in methanol and benzene extracts. Complete mortality was shown by methanol and benzene extract against *C. quinquefasciatus*. The methanol extract showed complete ovicidal activity against *A. aegypti*<sup>40</sup>.

#### Contact toxicity

The contact toxicity of leaf extracts was in the order *A. cocculus* (382.5 µg/mL), *C. halicacabum* (110 µg/mL), *C. laurifolius* (92.5 µg/mL) and *S. nux-vomica* (8.75 µg/mL) (Fig. 4a). In case of bark extracts, *S. nux-vomica* (137.5 µg/mL) had shown the maximum toxicity followed by *C. halicacabum* (87.5 µg/mL), *C. laurifolius* (15.25 µg/mL) and *A. cocculus* (11.25 µg/mL) (Fig. 4b). Acetone extracts of *Anamirta cocculus* fruit reported to show larvicidal activity against different instars of *Culex pipiens*<sup>41</sup>. Many of the compounds identified in the assessed plants have anti-insect activities. For example, sesquiterpenes like caryophyllene oxides are reported to have anti-termite activity<sup>42</sup>. This is in addition to the anti-inflammatory and cytotoxic activities exhibited by caryophyllene class of compounds<sup>43,44</sup>.

#### GC-MS analysis

Identification of chemical constituents was established based on the molecular structure, molecular mass and calculated fragments. Explication on GC-MS spectrum was conducted using the database NIST and Wiley library. The name, retention time, area and the base m/z of the components of the test materials were ascertained. The correlative percentage amount of each component was calculated by comparing its average peak area in the total area. The spectrum of the

unknown component was compared with the spectrum of the component in the library.

The identified phytochemical constituents in *A. cocculus* leaf extracts are neophytadiene, methylpalmitate, 4-nonenic acid-methyl ester, T-phytol, stigmasterol, gamma-sitosterol, gamma-curcumene, methyl 8,11,14-eicosatrienoate, Urs-12-ene, lupeol, ethyl iso-allocholate, squalene, longifolenaldehyde, (+)-trans-1-Isopropenyl-4-methyl-1,4-cyclohexanediol, (-)-Globulol and dodecanedioic acid. The bark extract contains coumarin, p-Vinylguaiaicol, pyrogallol dimethylether, neophytadiene, methyl palmitate, linoleic acid- methyl ester, 9-Octadecenoic Acid (Z)-methyl ester, phytol, methyl stearate, myo-Inositol, L-serine-ethyl ester, galactopyranoside, 3,6,9,12,15-Pentaoxonadecan- 1-ol, butyraldehyde-semicarbazone, levoglucosan, alpha-l-rhamnopyranose, 5-Methyl-2-hexanone oxime, N-Isoamylacetamide, methyl pentofuranoside, inositol, 1-deoxy, glycerol .beta.- palmitate, 1,2-enzenedicarboxylic Acid and methyl lignocerate (Table 1).

The leaf extract of *C. halicacabum* contains tridecyl acrylate, phytol acetate, hexahydrofarnesol, methyl palmitate, linolelaidic acid-methyl ester, 8,11,14-docosatrienoic acid methyl ester, phytol, methyl stearate, ethyl elaidate, ethyl 9,12-hexadecadienoate, eicosanoic acid -methyl ester, ethyl margarate, methyl melissicate, squalene, gamma-tocopherol and 3-bromocholest-5 ene. Phytochemical constituents in *C. halicacabum* bark methanol extracts are beta - caryophyllene epoxide, tridecyl acrylate, phytol acetate, 2-hydroxyhexadecyl butyrate, mome- inositol, methyl palmitate, linolelaidic acid-methyl ester, 2-methyltetracosane, phytol, eicosane, pentacosane and 2-methyloctacosane. Their retention time (RT), peak area in percentage, name and m/z ratio and chemical nature is shown in Table 2.

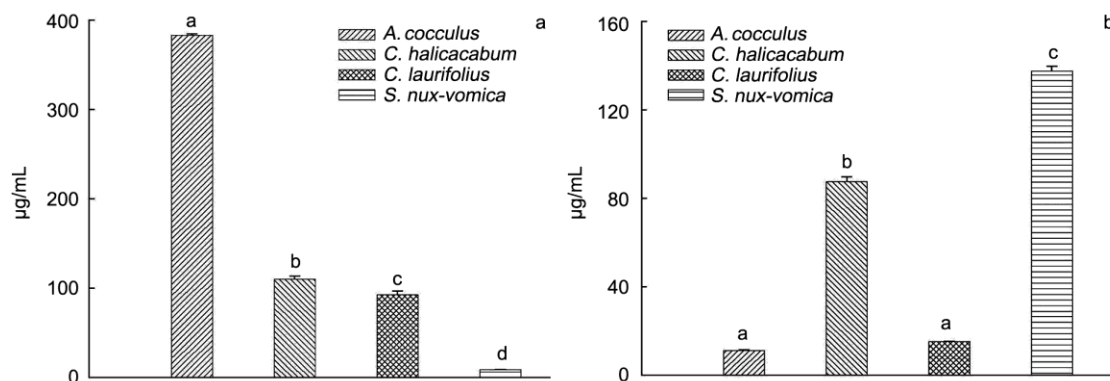


Fig. 4 — Contact toxicity of leaf (a) and bark (b) methanol extracts of *A. cocculus*, *C. halicacabum*, *C. laurifolius* and *S. nux-vomica* against *Spodoptera litura*. The values are expressed as mean  $\pm$  SE of four replicates per experiment. Means followed by different alphabets are significantly different at  $p \leq 0.05$  by Student-Newman-Keul's multiple comparison test.

Table 1 — Phytochemical constituents *A. cocculus* extracts

No	Retention Time	Area (%)	Name	Base m/z	Chemical Nature
Leaf					
1	26.542	12.80	Neophytadiene	68.10	Sesquiterpeninoids
2	28.765	14.88	Methylpalmitate	74.05	Saturated fatty acids methyl ester
3	31.875	1.29	4-Nonenoic acid, methyl ester	96.05	Fatty acids methyl ester
4	32.042	8.42	T-Phytol	71.10	Diterpene alcohol
5	34.416	11.93	Stigmasterol	55.05	Tetracyclitriterpinoids
6	39.835	19.47	Gamma-Sitosterol	55.05	Tetracyclitriterpinoids
7	41.725	1.91	Gamma-Curcumene	77.00	Sesquiterpene
8	42.054	2.05	Methyl 8,11,14-Eicosatrienoate	82.10	Methyl ester
9	42.150	2.96	Urs-12-ene	218.20	Diterpene
10	42.322	3.10	Lupeol	94.10	Pentacyclic terpenoids
11	42.892	0.94	Ethyl iso-allocholate	59.95	-----
12	43.051	0.97	Squalene	69.10	Pentacyclic triterpenoids
13	44.209	6.00	Longifolenaldehyde	138.15	Tricyclic sesquiterpene
14	45.400	1.16	(+)-trans-1-Isopropenyl-4-methyl-1,4-cyclohexanediol	108.10	Diol
15	45.501	3.89	(-)-Globulol	95.10	Sesquiterpenoids
16	45.617	0.76	Dodecanedioic Acid	52.95	Saturated fatty acids/Carboxylic acid
Bark					
1	12.300	0.48	Coumaran	120.10	Phenylpropanoids
2	14.209	1.31	p-Vinylguaiacol	150.05	2-Methoxy 4-Vinyl Phenol
3	15.192	3.27	Pyrogallol Dimethylether	154.10	-----
4	26.470	0.39	Neophytadiene	68.05	Sesquiterpenoids
5	28.290	0.89	Methyl palmitate	74.05	Saturated fatty acids methyl ester
6	31.487	0.35	Linoleic Acid, Methyl Ester	67.05	Saturated fatty acids methyl ester
7	31.615	0.81	9-Octadecenoic Acid (Z)-, Methyl Ester	55.05	Saturated fatty acids methyl ester
8	31.823	0.40	Phytol	71.05	Diterpene alcohol
9	32.125	0.44	Methyl stearate	74.05	Saturated fatty acids methyl ester
10	32.302	0.50	Myo-Inositol	73.05	Vitamin-B
11	32.367	1.60	L-Serine, Ethyl Ester	60.00	-----
12	32.417	2.56	Galactopyranoside	61.00	Galactoside
13	32.525	7.79	3,6,9,12,15-Pentaoxonadecan-1-ol	57.00	-----
14	32.717	5.04	Butyraldehyde, Semicarbazone	60.00	Aldehyde
15	32.758	10.48	Levoglucofan	60.00	Anhydrohexose
16	32.908	17.40	Alpha-l-rhamnopyranose	60.00	-----
17	33.117	5.59	5-Methyl-2-hexanone oxime	73.05	-----
18	33.308	0.70	N-Isoamylacetamide	73.05	-----
19	33.350	10.73	Methyl pentofuranoside	73.00	-----
20	33.547	25.61	Inositol, 1-deoxy-	73.05	1,2,3,4,5-Cyclohexanpentol
21	38.700	2.54	Glycerol .beta.- palmitate	57.05	Monoacylglyceride (Saturated fatty acids)
22	38.900	0.55	1,2-enenedicarboxylic Acid	149.00	Aromatic dicarboxylic acid (Phthalic Acid)
23	41.862	0.59	Methyl Lignocerate	74.05	Lignoceric acid Methyl ester

Methanol extracts of *C. laurifolius* bark had shown the presence of 24 compounds, however, the leaf extract had shown only 10 compounds. The compounds in bark extract were methoxyeugenol, hexahydrofarnesol, 3,5-dimethoxy-4-hydroxyphenyl acetic acid, neophytadiene, cholesterol dimethylsilyl

ether, methyl palmitate, inositol, 1- deoxy, methyl octadeca-9,12-dienoate, 9-octadecenoic acid (Z)-methyl ester, trans-13-octadecenoic acid-methyl ester, phytol, methyl stearate, ambrettolide, 4,8,12,16-tetramethylheptadecan-4-olide, 3,5,6-trimethyl-4-phenyl-2-pyridone, crinan-3-one, methyl docosanoate,

Table 2 — Phytochemical constituents *C. halicacabum* extracts

No	Retention Time	Area (%)	Name	Base m/z	Chemical Nature
Leaf					
1	23.585	7.24	Tridecyl acrylate	55.05	Ester
2	26.652	15.68	Phytol, acetate	68.10	Diterpene alcohol
3	26.772	3.35	Hexahydrofarnesol	70.10	Sesquiterpenoids
4	28.472	10.83	Methyl Palmitate	74.10	Saturated fatty acids methyl ester
5	31.674	3.40	Linolelaidic Acid, Methyl Ester	67.10	Poly unsaturated fatty acid methyl ester
6	31.797	12.08	8,11,14-Docosatrienoic Acid, Methyl Ester	55.10	Fatty acid Methyl ester
7	32.021	17.58	Phytol	71.10	Diterpene alcohol
8	32.304	2.96	Methyl stearate	74.05	Saturated fatty acids methyl ester
9	33.147	1.17	Ethyl elaidate	55.10	Elaidic acid ethyl ester
10	34.631	3.39	Ethyl 9,12-hexadecadienoate	67.10	Saturated Fatty acid methyl ester
11	35.821	1.02	Eicosanoic Acid, Methyl Ester	74.10	Saturated fatty acids methyl ester
12	36.528	5.11	Ethyl Margarate	57.10	Saturated fatty acids ethyl ester
13	39.075	1.07	Methyl Melissicate	74.10	Very long chain fatty acids
14	43.223	6.94	Squalene	69.10	Pentacyclic triterpenoids
15	46.963	1.57	Gamma-Tocopherol	151.15	Vitamin-E
16	47.636	1.74	3-Bromocholest-5-ene	57.10	-----
Bark					
1	21.055	1.39	Beta - Caryophyllene Epoxide	79.10	Bicyclic sesquiterpene
2	23.518	3.41	Tridecyl acrylate	55.05	-----
3	26.597	2.13	Phytol acetate	68.10	Diterpene alcohol
4	27.117	1.23	2-Hydroxyhexadecyl butyrate	73.05	-----
5	27.217	3.93	Mome Inositol	87.10	-----
6	28.414	3.67	Methyl Palmitate	74.10	Saturated fatty acids methyl ester
7	31.613	2.57	Linolelaidic Acid, Methyl Ester	67.10	Poly unsaturated fatty acid methyl ester
8	31.764	5.33	2-Methyltetracosane	57.10	Alkane
9	31.950	4.93	Phytol	71.10	Diterpene alcohol
10	35.308	8.95	Eicosane	57.10	Alkane
11	41.579	7.64	Pentacosane	57.10	Hydrocarbon
12	47.810	1.19	2-Methyloctacosane	57.10	Alkane

benzoic acid, 4-(3-acetoxy-4-methoxybenzylideneamino)-1 methyl ester, 1H-naphtho[2,1-b]pyran-1-one,7,8-dimethoxy-2-methyl-,2-chloro-4,5methylenedioxyamphet- amine, vinyl methyl ether, dibenz[d,f]cycloheptanone, 2,3,9-trimethoxy-, gamma-tocopherol and vitamin E. The leaf extracts contain, phytol-acetate, hexahydrofarnesylacetone, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, methyl palmitate, methyl elaidate, phytol, 4,6-dimethyl-1,4,6-oxadiazocane-5-Thione, palmitaldehyde-diallyl acetal and pentacosane (Table 3).

The *S. nux-vomica* bark extracts contain methaqualone metabolite VI (hypnotic), chinasaur, methoxyeugenol, 5-ethyl-2-nonanol, methyl palmitate, mome inositol, 1,3-oxathiane, 5-isopropyl-2-methyl, ethyl pentofuranoside, 9,12-octadecadienoic acid -methyl ester, 9-octadecenoic Acid (Z)-methyl ester and gamma-sitosterol. The leaf extracts contain,

methaqualone metabolite VI (hypnotic), neophytadiene, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, phytol-acetate, methyl palmitate, mome inositol, phytol, glyoxal, 1H-purin-6-amine, [(2-Fluorophenyl) methyl] and strychnidin-10-one (Table 4).

The essential oil from the rhizomes of *Zingiber zerumbet* had shown contact and repellent activities because of the presence of caryophyllene and other molecules in the essential oils<sup>45</sup>. A study conducted on the essential oils from different genotypes of citrus from Brazil had shown the repellent activity against *Diaphorina citri* Kuwayama and the presence of phytol in these extracts were also confirmed by GC-MS analysis<sup>46</sup>. Essential oils of *Mentha longifolia*, *Pulicaria gnaphalodes* and *Achillea wilhelmsii* had shown insecticide activity against two stored product pests, the flour beetle, *Tribolium castaneum*, and the cowpea weevil, *Callosobruchus maculatus*. Further



Table 3 — Phytochemical constituents of *C. laurifolius* extracts

No	Retention Time	Area (%)	Name	Base m/z	Chemical Nature
Leaf					
1	26.486	27.85	Phytol, acetate	68.10	Diterpene alcohol
2	26.613	16.44	Hexahydrofarnesylacetone	58.05	-----
3	27.365	8.35	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	82.10	-----
4	28.357	6.88	Methyl Palmitate	74.05	Saturated fatty acids Methyl esters
5	31.652	6.04	Methyl Elaidate	85.05	Unsaturated trans fatty acid
6	31.849	11.31	Phytol	71.05	Diterpene alcohol
7	32.183	8.40	4,6-Dimethyl-1,4,6-Oxadiazocane-5-Thione	174.15	-----
8	32.409	2.83	Palmitaldehyde, Diallyl Acetal	84.10	-----
9	44.243	3.65	Pentacosane	57.05	Hydrocarbon
Bark					
1	23.724	15.59	Methoxyeugenol	194.05	-----
2	24.155	0.77	Hexahydrofarnesol	56.05	Sesquiterpenoids
3	25.251	4.84	3,5-Dimethoxy-4-hydroxyphenyl acetic acid	167.05	-----
4	26.473	0.60	Neophytadiene	68.05	Sesquiterpenoids
5	27.478	0.74	Cholesterol Dimethylsilyl Ether	75.05	Sterol
6	28.298	1.71	Methyl palmitate	74.05	Saturated fatty acids Methyl ester
7	29.592	0.54	Inositol, 1- deoxy	73.05	-----
8	31.492	2.81	Methyl Octadeca-9,12-dienoate	67.05	Fatty acid methyl ester
9	31.617	2.04	9-Octadecenoic Acid (Z)-, Methyl Ester	55.00	Saturated fatty acids Methyl ester
10	31.730	0.63	Trans-13-Octadecenoic acid, methyl ester	55.00	Saturated fatty acids Methyl ester
11	31.822	0.64	Phytol	71.05	Diterpene alcohol
12	32.117	0.42	Methyl stearate	74.05	Saturated fatty acids methyl ester
13	32.447	0.92	Ambrettolide	55.05	-----
14	35.985	0.78	4,8,12,16-Tetramethylheptadecan-4-olide	99.05	-----
15	36.646	3.60	3,5,6-Trimethyl-4-Phenyl-2-Pyridone	212.05	-----
16	38.817	1.52	Crinan-3-one	271.10	-----
17	38.877	1.56	Methyl docosanoate	74.05	Saturated fatty acids
18	40.316	6.76	Benzoic acid, 4-(3-acetoxy-4-methoxybenzylideneamino)-1 methyl ester	285.10	-----
19	42.106	17.44	1H-Naphtho[2,1-b]pyran-1-one, 7,8-dimethoxy-2-methyl-	270.05	-----
20	42.192	16.59	2-chloro-4,5-methyleendioxyamphetamine	58.05	-----
21	42.592	1.74	Vinyl Methyl Ether	58.05	Enol ether
22	44.736	14.35	Dibenz[d,f]cycloheptanone, 2,3,9-trimethoxy-	298.10	-----
23	46.626	1.16	gamma-Tocopherol	151.10	Tocopherol
24	48.105	2.26	Vitamin E	165.10	Tocopherol

analysis of the oils had shown the presence of eicosane along with many other compounds<sup>47</sup>. n-Pentacosane is reported to act as both contact and volatile pheromone in the tea weevil, *Myllocerinus*

*aurolineatus*<sup>48</sup>. It was reported in a recent study that squalene showed repellent against whitefly adults, whitefly nymphal toxicity and mite toxicity<sup>49</sup>. Insecticidal activity of *Jatropha curcas* against

Table 4 — Phytochemical constituents of *S. nux-vomica* extracts

No	Retention Time	Area (%)	Name	Base m/z	Chemical Nature
Leaf					
1	18.049	2.63	Methaqualone Metabolite VI (Hypnotic)	251.00	Quinazolines
2	26.476	14.05	Neophytadiene	68.05	Sesquiterpenoids
3	26.984	3.08	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	82.05	-----
4	27.357	5.54	Phytol, acetate	82.10	Diterpene alcohol
5	28.300	10.20	Methyl palmitate	74.05	Saturated fatty acids methyl ester
6	31.499	31.57	Mome Inositol	73.00	-----
7	31.825	15.87	Phytol	71.05	Diterpene alcohol
8	32.678	3.08	Glyoxal	58.00	Dialdehyde
9	45.417	4.89	1H-Purin-6-Amine, [(2-Fluorophenyl)Methyl]-	73.05	-----
10	49.231	9.08	Strychnidin-10-one	334.10	Alkaloid
Bark					
1	18.043	0.79	Methaqualone Metabolite VI (Hypnotic)	251.05	-----
2	23.233	4.97	Chinasaur	60.00	Cyclitol, Cyclicpolyol, Cyclohexanecarboxylic acid
3	23.867	6.53	Methoxyeugenol	194.05	-----
4	24.142	5.31	5-Ethyl-2-Nonanol	57.00	-----
5	28.298	1.28	Methyl palmitate	74.05	Saturated fatty acids Methyl esters
6	29.643	4.24	Mome Inositol	73.05	-----
7	29.733	4.73	1,3-Oxathiane, 5-isopropyl-2-methyl-	75.05	-----
8	29.875	10.01	Ethyl pentofuranoside	60.00	-----
9	31.494	0.61	9,12-Octadecadienoic acid, methyl ester	67.05	Saturated fatty acids Methyl esters
10	31.619	1.11	9-Octadecenoic Acid (Z)-, Methyl Ester	55.05	Saturated fatty acids Methyl esters
11	39.826	1.83	gamma-Sitosterol	55.05	Tetracyclic triterpenoids

housefly, *Musca domestica* could be due to the presence of trans-phytol and squalene in the extracts<sup>50</sup>. Studies were shown that toxicity and larvicidal activity of the essential oil from *Acalypha segetalis* could be due to the presence of the major components alpha-pinene, neophytadiene, isomer II and neophytadiene, isomer III<sup>51</sup>.

### Conclusion

*S. litura* being a pest which causes economic damage to many of the vegetables and important crops, effective mechanisms are required for controlling them. Due to the continuous and irrational use of chemical agents, the pest has become resistant to many of them and the incessant use of chemical agents are becoming more dangerous to non-target pests and other environmental factors. Through the present study, it was found that effective anti-feedant and repellent agents or formulations could be developed from *S. nux-vomica*, *C. halicacabum* and *C. laurifolius*, which contain many phytochemicals with proven anti-insect properties.

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### Conflict of interest

The authors declare that they have no conflict of interest.

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