



**Effect of *Duranta erecta* Linn (Verbenaceae) leaf extracts against Armyworm *Spodoptera litura* and Cotton bollworm *Helicoverpa armigera* (Lepidoptera: Noctuidae)**

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**Abstract**

In the present study, petroleum ether, chloroform and ethyl acetate crude extracts of *Duranta erecta* leaves were investigated for their antifeedant, larvicidal, pupicidal, oviposition deterrent, ovicidal and growth inhibition activities against *Spodoptera litura* and *Helicoverpa armigera*. Ethyl acetate extracts of *D. erecta* showed higher percentage of antifeedant, larvicidal, pupicidal and ovicidal activities. Moreover, the highest percentage of deformed larvae, pupae and adults were found in ethyl acetate extract. Fewer adults successfully emerged in ethyl acetate extract in comparison with the control and the other extracts. Preliminary phytochemical analysis showed the presence of Terpenoids, Coumarin and Phenols in ethyl acetate extract. Plant derived chemicals offer a more natural and environmentally friendly approach to agricultural pest control as an alternative to chemical pesticides. Further, the active compounds will be isolate from the ethyl acetate extracts which will be useful for controlling economically important insect pests.

**Keywords:** Antifeedant, *Duranta erecta*, *Helicoverpa armigera*, Larvicidal, *Spodoptera litura*.

**1. Introduction**

The Asian armyworm, *Spodoptera litura* (Fabricius) is a polyphagous insect pest of cosmopolitan distribution for about 150 host species [1, 2] This insect pest plays a major role in damaging the agriculture crops. It causes economic losses to many vegetables and field crops from 10 to 30% [3]. The cotton bollworm, *Helicoverpa armigera* (Hubner) is a another polyphagous pest of worldwide occurrence inflicting crop damage in India to the sum of one billion dollars annually and it attacks over 200 crop species belonging to 45 families [4,5]. It is also widely distributed in India and attacks varied plant species, leading to considerable losses [6]. In India this insect

occurs as a major pest in many economically important crop including cotton, pigeonpea, chickpea, tomato, okra and blackgram. Bollworms are relatively safe from natural enemies because of the cryptic feeding habits of the larvae within cotton bolls. This pest is considered as the major damaging lepidopteran species [7]. The ability of these insect pests to thrive on alternate host plants is an adaptive advantage for its survival.

In recent years, Continuous use of synthetic organic insecticides in crop pest control programs around the world has resulted in damage to the environment, toxic

to natural enemies, pest resurgence and gradually the pest will develop resistance to it [8-10]. Plant derived pesticide offer a more natural 'Environmentally friendly' approach to pest control than synthetic insecticides [11]. Plants are a rich source of organic chemicals on earth. Already more than 10,000 secondary metabolites have been chemically identified. In nature many plants have unpalatable substances like high content of phenols, alkaloids, flavanoids, terpenes, quinone, coumarin etc., which play a defensive role against particularly agriculture insect pests. Identifying sources with useful biological activity is only the starting point in the long process of development of a botanical pest management product. Success of botanical in the field depends on number of factors such as, ongoing availability of the natural resources, adequate biomass to justify extraction, the feasibility of extraction near the harvest site and the stability of the extract in storage after preparation [12]. Plant-based pesticides are highly suitable since they have low toxicity, are easily biodegradable, and have multimode of action [13].

*Duranta erecta* Linn (Verbenaceae) is a shrub, growing in disturbed areas in moist habitat, Golden dewdrop, also known as skyflower, pigeon berry, angels-whisper, also called Katamehedi. [14]. Insecticidal property of methanol and aqueous extracts of *D. erecta* leaves tested against larvae of *Culex quinquefasciatus* showed significant larval mortality [15]. However, this plant has no report on biological properties against agricultural insect pests. Hence, in the present investigation we evaluate the biological activity of *D. erecta* against economically important insect pests.

## 2. Materials and Methods

### 2.1. Collection and extraction of plant material

The plant of *Duranta erecta* Linn (Verbenaceae) leaves were collected from Dhalavai halli, Dharmapuri District, during the November and December, 2014. The plant specimen was identified by Dr. S. John Britto, Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College, Tiruchirappalli District, Tamil Nadu, India. The leaves were thoroughly washed with tap water and shade dried under room temperature (27.0°C ±2.0) at PG & Research Department of Zoology, Government Arts College, Musiri, Tamil Nadu, India. After complete drying the leaves were powdered using electric blender and sieved through kitchen strainer (Medium sieve: mesh size = 1/16 inch). 1 Kg of plant powder

was extracted with one third of petroleum ether, chloroform and ethyl acetate. Then, each solution was filtered through Whatman's No.1 filter paper. The solvents from the crude extracts were evaporated to air dryness at room temperature for until complete dryness of solvent. The crude extracts were collected in clean Borosil vials and stored in the refrigerator at 4 °C for subsequent bioassay against *S.litura* and *H.armigera*. The voucher specimen (IPH No.51) was prepared and deposited at PG & Research Department of Zoology, A. A. Government Arts College, Musiri, Tamil Nadu, India.

### 2.2. Rearing of test organisms

Egg mass of *S. litura* and different larval stages of *H.armigera* were collected from vegetable field at Anaipatti, Musiri, Trichy, Tamil Nadu, India. Larvae were reared in laboratory conditions (27.0°C ± 2°C; 70 % RH) throughout the study period at PG & Research Department of Zoology, Government Arts College, Musiri, Tamil Nadu, India.

### 2.3. Antifeedant activity

Antifeedant activity of crude extracts of *D. erecta* was studied using leaf disc no-choice method [16]. Polysorbate 20 (Tween 20) was used as emulsifier at 0.05% [17]. Fresh cotton leaf (for *H.armigera*) and castor leaf (for *S.litura*) discs of 3 cm diameter were punched using cork borer and dipped in 0.625%, 1.25%, 2.50% and 5.00% extracts separately and air dried for 5 minutes. After air drying, treated leaf discs were kept inside the Petri dishes (15mm × 90 mm diameter) separately containing wet filter paper to avoid drying of the leaf disc. 4<sup>th</sup> instar *H. armigera* and *S. litura* new emerged larvae were selected and starved for 2 hours. Then, one larva was introduced on each treated leaf disc. Leaf discs treated with acetone were considered as control. Ten replications were maintained for each treatment. Progressive consumption of leaf area by the larva in 24 h period was recorded in control and treatments using leaf area meter (Systronics 211). Leaf area consumed in plant extract treatment was corrected from the control. The percentage of antifeedant index (AI) was calculated using the formula of [18].

$$AI = [(C - T) / (C + T)] \times 100$$

Where

C and T represent the amount of leaf eaten by the larva on control and treated discs respectively.

## 2.4. Larvicidal activity

Each crude extract was dissolved in acetone to obtain the final concentrations of 0.625%, 1.25%, 2.50% and 5.00%. Fresh cotton leaf (for *H.armigera*) and castor leaf (for *S. litura*) were treated with different concentrations of crude extracts. Leaf discs with acetone were considered as control. Petioles of the leaves were tied with wet cotton plug (to avoid early

drying) and placed in round plastic trough (29 cm × 8 cm). In each concentration 10 pre-starved (2 hrs) fourth instar larvae of *S. litura* and *H. armigera* were introduced individually and covered with muslin cloth. Five replicates were maintained for all concentrations and the number of dead larvae was recorded after 24 h up to pupation. Percentage of larval mortality was calculated and corrected by Abbott's [19].

$$\text{Abbott's percent corrected mortality} = \frac{\% \text{ Mortality in treated} - \% \text{ Mortality in control}}{100 - \% \text{ Mortality in control}} \times 100$$

## 2.5. Growth inhibition activity

Growth regulation activity of crude extracts was studied at four different concentrations (as mentioned in antifeedant activity) against fourth instar larvae of *S.litura* and *H. armigera*. Ten larvae were introduced in a Petri-plate containing tomato leaves treated with different concentrations of crude extracts. Acetone treated leaves were considered as control. After 24 hrs of feeding, the larvae were transferred to normal leaves for studying the developmental period. For each concentration five replicates were maintained. During the developmental period, deformed larvae, pupae, adults and successful adults emerged were recorded.

## 2.6. Pupicidal activity

Ten newly pupated *H. armigera* and *S.litura* 2 days old were separated and dipped in various concentrations (as mentioned in antifeedant activity) for 5 minutes. Five replicates were maintained (n=100). Control were used as acetone. Pupal mortality was calculated by subtracting the number of emerging adults from the total number of pupae. During the developmental period, pupal duration, fecundity and hatchability were recorded.

## 2.7. Ovicidal activity

Twenty individual eggs of *H. armigera* and *S.litura* were separated using brush and dipped in various concentrations (as mentioned in antifeedant activity) for few minutes. Acetone used as control. Five replicates were maintained (n=100). Number of eggs hatched in the control and treatments were recorded and percent ovicidal activity was calculated according to Abbott's [19] formula (as mentioned in larvicidal activity).

## 2.8. Data Analysis

Data analysis was carried out using Microsoft Excel 2007. Two-way ANOVA was performed for all the experimental data from that Least Significant Difference was calculated and the significant differences were marked with different alphabet.

## 3. Results

The results of the antifeedant potential of the solvent crude extracts of *D. erecta* investigated against *S. litura* and *H. armigera* larvae were presented in Table 1. Antifeedant activity was assessed based on antifeedant index. Higher antifeedant index normally indicates decreased rate of feeding. In the present study, irrespective of concentration and solvents used for extraction the antifeedant activity varied significantly. Data pertaining to the above experiment clearly revealed that maximum antifeedant activity was recorded in ethyl acetate extract on *S. litura*(80.37%) and *H.armigera* (78.18%) at 5% concentration, followed by chloroform extract (%) and petroleum ether extract (%) at the same concentration. One-way analysis of variance followed by LSD test showed statistical significance (P<0.05).

Percentage larvicidal activity of *D. erecta*, studied at different concentrations against *S.litura* and *H. armigera* was presented in table 2. High larval mortality normally indicates potential larvicidal activity of plant extracts. In the present study irrespective of concentration and solvents used for extraction, the insecticidal activity varied significantly. Insecticidal activity data revealed clearly that maximum insecticidal activity was recorded in ethyl acetate extract on *S. litura* (69.88%) and *H. armigera* (63.2%). One-way analysis of variance (ANOVA) followed by least significant difference (LSD) test showed statistical significance (p < 0.05) compared to control.

**Table 1.** Antifeedant activity (%) of crude extracts of *D. erecta* against fourth instar larvae of *S. litura* and *H. armigera*.

Solvent extracts	<i>S.litura</i>				<i>H.armigera</i>			
	Concentration tested (%)							
	0.625	1.25	2.5	5	0.625	1.25	2.5	5
Petroleum ether	10.48±2.5 <sup>b</sup>	21.64±4.7 <sup>b</sup>	33.66±3.3 <sup>b</sup>	43.07±4.1 <sup>b</sup>	12.32±4.5 <sup>b</sup>	20.49±4.9 <sup>b</sup>	31.86±5.2 <sup>b</sup>	40.44±5.5 <sup>b</sup>
Chloroform	17.94±6.4 <sup>c</sup>	29.41±5.9 <sup>c</sup>	42.13±6.8 <sup>c</sup>	62.34±4.6 <sup>c</sup>	14.42±2.7 <sup>b</sup>	26.75±4.5 <sup>b</sup>	33.72±2.9 <sup>b</sup>	59.37±5.0 <sup>c</sup>
Ethyl acetate	24.27±5.1 <sup>d</sup>	40.72±6.3 <sup>d</sup>	57.89±4.4 <sup>d</sup>	80.37±4.2 <sup>d</sup>	21.0±4.8 <sup>d</sup>	36.32±3.1 <sup>c</sup>	53.18±4.1 <sup>c</sup>	78.18±2.5 <sup>d</sup>

Values are mean of ten replications. Within the column similar alphabets are statistically not significant (p > 0.05 by LSD).

**Table 2.** Insecticidal activity (%) of crude extracts *D. erecta* against third instar larvae of *S. litura* and *H. armigera*.

Solvent extracts	<i>S. litura</i>				<i>H. armigera</i>			
	Concentration tested (%)							
	0.625	1.25	2.5	5	0.625	1.25	2.5	5
Petroleum ether	9.34±4.5 <sup>b</sup>	17.9±5.1 <sup>b</sup>	22.42±5.4 <sup>b</sup>	29.7±5.8 <sup>b</sup>	7.23±4.6 <sup>b</sup>	13.30±5.2 <sup>b</sup>	19.27±3.2 <sup>b</sup>	24.35±4.5 <sup>b</sup>
Chloroform	14.18±4.6 <sup>b</sup>	21.29±5.6 <sup>b</sup>	27.46±5.7 <sup>b</sup>	38.0±4.2 <sup>b</sup>	12.32±4.5 <sup>b</sup>	20.49±4.9 <sup>b</sup>	25.86±5.2 <sup>b</sup>	34.44±5.5 <sup>c</sup>
Ethyl acetate	21.73±6.4 <sup>c</sup>	38.62±6.2 <sup>c</sup>	50.78±7.1 <sup>c</sup>	69.88±2.4 <sup>c</sup>	19.4±1.3 <sup>b</sup>	35.2±2.1 <sup>c</sup>	47.00±1.2 <sup>c</sup>	63.2±3.3 <sup>d</sup>

Values are mean of five replications. Within the column similar alphabets are statistically not significant (p > 0.05 by LSD).

Percentage of deformities due to the treatment of crude extracts from leaves of *D. erecta* at 5% concentration was presented in Table-3 and Figure-1&2. Highest larval, pupal and adult deformities were observed in ethyl acetate extract on both insects. The deformed adult moths were recognized by their

relatively poor body size, highly curled wings and under grown wings. The lowest percentage of successful adult emergence was found in ethyl acetate extract with 27.71% and 31.70% on *S. litura* and *H. armigera* respectively.

**Table 3.** Percentage of Mortality (%) and successful adult emergence (%) of *S. litura* and *H. armigera* fed on *D. erecta* crude extract at 5% concentration.

Solvent extracts	<i>S. litura</i>				<i>H. armigera</i>			
	Larvae dead	Pupae dead	Adult dead	Successful adult emergence	Larvae dead	Pupae dead	Adult dead	Successful adult emergence
Petroleum ether	5.08	8.02	10.07	76.83	4.85	5.77	6.45	82.93
Chloroform	13.50	16.00	16.22	54.28	11.60	14.07	17.6	56.73
Ethyl acetate	25.35	20.62	26.32	27.71	20.82	17.60	22.86	31.70
Control	1.3±1.1			98.7	0.9±1.0			99.1

Values are mean ± Standard Deviation of five replications



**Figure 1.** Different malformations in *S. litura* due to the treatments of *D. erecta* extracts. A- Control larvae; B- Abnormal larvae; C- Control pupa; D- Dead pupae; E- Normal adult; F- Abnormal adult.



**Figure 2.** Different malformations in *H. armigera* due to the treatments of *D. erecta* extracts. A- Normal larvae; B- Abnormal larvae; C- Normal pupa; D- Larval and pupal intermediate; E & F- Abnormal adult

Pupicidal activity of *D. erecta* against *S. litura* and *H. armigera* was studied at 5% concentration. Highest pupal mortality indicates potential pupicidal activity of plant extracts. Significant pupicidal activity was

recorded in ethyl acetate extract on both insects. In addition high percentage of pupal duration, fecundity, hatchability in the same extract compared to control and other extracts (Table 4).

**Table 4.** Pupicidal activity of *D. erecta* crude extract against *S. litura* and *H. armigera* at 5% concentration .

Solvent extracts	<i>S. litura</i>				<i>H. armigera</i>			
	Pupal mortality (%)	Pupal duration (days)	Fecundity (No. of egg /moth)	Hatchability (%)	Pupal mortality (%)	Pupal duration (days)	Fecundity (No. of egg /moth)	Hatchability (%)
Petroleum ether	8.22±0.9	12.89±0.4	1037.64±6.0	18.67±1.8	2.54±1.4	9.21±0.6	798.34±5.2	12.45±5.6
Chloroform	14.65±3.2	14.23±1.0	706.03±3.9	43.00±2.0	12.79±4.6	11.90±1.0	594.78±3.1	40.99±4.2
Ethyl acetate	22.00±4.5	16.95±1.7	345.56±4.7	65.40±5.2	19.02±3.2	13.55±0.2	231.56±4.7	59.33±2.7
Control	1.8±1.3	11.6±1.2	1198.34±5.0	99.8±0.0	1.4±1.1	8.50±0.5	932.17±6.8	99.4±1.2

Values are mean ± Standard Deviation of five replications

Ovicidal activity of leaves extract of *D. erecta* against selected insect pests at four different concentrations was presented in table 5. Ovicidal activity indicates mortality of eggs by potential active principles present in the particular extracts. In the present study, maximum ovicidal activity against *S. litura* (72.04%) and *H. armigera* (68.96%) was recorded in ethyl acetate extract at 5% concentration.

One-way analysis of variance followed by LSD test showed statistical significance (P<0.05) compared to control. Further, ethyl acetate extract was subjected to preliminary phytochemical analysis for the confirmation of major group of compounds. Extracts showed positive results for the presence of coumarin, phenols and terpenoids in the preliminary analysis of phytochemicals (Table 6 and Figure-3).

**Table 5.**Ovicidal activity (%) of *D.erecta* against *S. litura* and *H. armigera*.

Solvent extracts	<i>S. litura</i>				<i>H. armigera</i>			
	Concentration tested (%)							
	0.625	1.25	2.5	5	0.625	1.25	2.5	5
Petroleum ether	9.36±1.4 <sup>a</sup>	14.54±3.0 <sup>b</sup>	21.82±2.2 <sup>b</sup>	30.82±4.2 <sup>b</sup>	8.60±2.7 <sup>a</sup>	12.70±3.3 <sup>b</sup>	18.66±4.0 <sup>b</sup>	24.92±3.9 <sup>b</sup>
Chloroform	15.87±6.0 <sup>ab</sup>	26.42±1.9 <sup>c</sup>	44.11±4.4 <sup>c</sup>	48.37±5.0 <sup>c</sup>	13.29±1.4 <sup>ab</sup>	23.92±6.6 <sup>c</sup>	32.5±4.5 <sup>c</sup>	39.64±2.9 <sup>c</sup>
Ethyl acetate	23.92±6.6 <sup>c</sup>	41.92±6.2 <sup>d</sup>	57.14±6.3 <sup>d</sup>	72.04±5.3 <sup>d</sup>	18.73±6.4 <sup>bc</sup>	37.59±4.8 <sup>d</sup>	51.92±6.2 <sup>d</sup>	68.96±3.2 <sup>d</sup>
Control	2.4±1.2 <sup>a</sup>				3.2±1.5 <sup>a</sup>			

Values are mean of five replications. Within the column similar alphabets are statistically not significant (p > 0.05 by LSD).

**Table-6:** Preliminary phytochemical analysis of ethyl acetate extracts of *D. erecta*.

Phytoconstituents	Name of test	Response	Results
Alkaloids	Mayers	Green colouration	-
Anthraquinones	Borntegers	Red colouration	-
Catechin	Salkowski	Pink colour	-
Coumarin	NaOH	Yellow colour	+
Flavonoids	Late acetate	Pure pink colour	-
Phenols	Late acetate	Intense colour	+
Quinines	Conc Hcl	Yellow colour	-
Saponins	Foam	Foam lather	-
Steroids	Salkowski	Green fluorescence	-
Tannins	Ferric chloride	White precipitate	-
Terpenoids	Salkowski	Purple colour	+

+ Presence of compound - Absence of compound



**Figure 3.** Phytoconstituents in ethyl acetate extract of *D.erecta*.

#### 4. Discussion

Plant based natural products in insect pest management programs are received much attention in recent years due to environmental pollution, pest resistance and resurgence, and undesirable effects to the nontarget organisms caused by unsystematic use of synthetic pesticides. Several plant extracts or isolated active compounds have been shown to possess antifeedant activity [20]. In many countries, plant derived products are being used by the farmers from

ancient times and it triggered the scientists to search for eco-friendly insecticides from plant kingdom. Some of compounds affect the feeding behavior of the insects and inhibit feeding. While few others disrupt hormonal balance by inhibiting the growth, metamorphosis and reproduction. Several hundred plants have been reported as insect repellents, antifeedants, attractants, insecticides, ovicides and oviposition deterrents [21-23].

The present study, ethyl acetate extract of *D. erecta* was promising in reducing feeding rate of selected economically important pests. Depending upon the concentration of the plant extracts the rate of feeding significantly varied. This indicates that the active principles present in the particular crude extracts inhibit larval feeding behavior by making the food unpalatable or by acting some substances directly on the chemosensilla of the larva resulting in feeding deterrence. These findings are agreement with the earlier reported that antifeedant and larvicidal activities of rhein isolated from *Cassia fistula* flower against lepidopteron pests *S. litura* and *H. armigera* [24]. Recently, it was reported that ethyl acetate extract of *Pseudocalymma alliaceum* showed significant antifeedant and insecticidal activities against selected pests [25]. Antifeedant chemicals play a major role in the unsuitability of non host plants as food for insects. Isolation and structure elucidation of these active chemicals is important not only for understanding the ecological aspects of insect pests relationship, but also for their potential in insect pests control [26].

Screening of plant extracts for deleterious effects on insects is one of the approaches used in the search for novel botanical insecticides [27]. In the present study ethyl acetate extract of *D. erecta* exhibited significant larvicidal activity at higher concentration (5%). It is possible that the insecticidal property present in the selected plant compound may arrest the various metabolic activities of the larvae during the development and ultimately the larvae failed to moult and finally died. In the present study, preliminary phytochemical analysis of leaves crude extract of *D. erecta* revealed that terpenoids, coumarin and phenols present in the ethyl acetate extracts. These compounds indicates the higher insecticidal potential of *D. erecta*. Present works agreed with earlier reports, Limonoid triterpenes are known to possess insecticidal and antifungal properties [28,29]. Phenolics are toxic to insects, fungi, bacteria, nematodes and weeds [30,31]. Similar works have already reported insecticidal activity of many plants and their compounds against different group of insects [32].

Insect growth regulation properties of plant extracts are very interesting and unique in nature, since insect growth regulator works on juvenile hormone. The enzyme ecdysone plays a major role in shedding of old skin and the phenomenon is called ecdysis or moulting. When the active plant compounds enter into the body of the larvae, the activity of ecdysone is suppressed and the larva fails to moult, remaining in the larval stage and ultimately dying [33]. In the present study maximum percentage of deformed

larvae, pupae and adults were noted in ethyl acetate extract treated larvae. The morphological deformities at larval, pupal and adult stages are due to toxic effects of crude extract on growth and development processes. Since morphogenetic hormones regulate these processes, it can be suggested that these solvent crude extracts interfere with these hormones of the insects. These results are consistent with the earlier reports on various lepidopteran species [34-36]. In the present study, ethyl acetate extract of *D. erecta* exhibited significant pupicidal activity at 5% concentration. These findings are agreement with the earlier reported that pupicidal activity of *Cleome viscosa* and *Sinapis alba* seed extracts against *H. armigera*. Methanol extract of *S. alba* seeds recorded the highest pupal mortality (54%) at 2% concentration [37]. Pupicidal activities of *Atalantia monophylla* against *H. armigera* [38].

Ovicidal activity of plant extracts is important to control the pest at eggs stage itself thereby preventing the damage caused by other stages. In the present study, ethyl acetate extracts showed maximum ovicidal activity. The hatchability of *S. litura* and *H. armigera* eggs was directly proportional to the concentration of plant extract. The results of the present study are in agreement with the earlier findings on the ovicidal effect of different plant origins. Elumalai *et al.* [39] noticed ovicidal activity in plant oils of *Zingiber officinale*, *Ocimum basilicum*, *Cyperus scariosus*, *Pimpinella anisum*, *Nigella sativa*, *Rosmarinus officinalis* and *Curcuma longa* against *S. litura*. Baskar *et al.* [40] also reported that crude and fractions from *Atalantia monophylla* leaves showed ovicidal activity against *S. litura*.

In the present study, preliminary phytochemical analysis revealed that Terpenoids, Coumarin and Phenols presents in the ethyl acetate extract indicates that higher percentage of biological activity. Alkaloids present in *Tylophora asthmatica* plant inhibited the feeding of *S. litura* [41]. Morimoto *et al.*, [42] who have reported that quinone, remirol and cyperquinone isolated from the plants of the family Cyperaceae had strong antifeedant activity against *S. litura*.

## 5. Conclusion

Ethyl acetate crude extract of *D. erecta* showed higher percent of phytopesticidal effects against *H. armigera* and *S. litura*. It is first time report on this agriculture field important insect pest. Further, it may be suggested that the active crude extract of *D. erecta* will be isolate and identify the effective compounds which will be used for controlling the economically important insect pests.

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## Competing interests

The authors of this work have no competing interests.

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