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# Toxic Effects of *Lantana camara* Crude Extracts on *Spodoptera litura* (Fabr.)

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*Lantana camara* flowers were extracted by Soxhlet apparatus for 8 h with *n*-hexane, dichloromethane and methanol, successively. Each crude extract of flowers was tested for topical toxicity by applying the extract on the third thorax of *Spodoptera litura* (second instar). Results showed that the 10 % (w/v) of dichloromethane crude extract was the most effective, causing 56 % of *S. litura* to die in 7 days. The 10 % of *n*-hexane extract caused 34 % death, which was higher than the 2 % death observed in controls ($p < 0.05$). The mean lethal concentration ($LC_{50}$) of the dichloromethane extract toward *S. litura* larvae was 9.453 % w/v. The dichloromethane and *n*-hexane crude extracts reduced feeding of the third instar of *S. litura*. There were no differences in larvae mortality among the three extracts when given orally. However, these experiments terminated at 7 days, so longer term effects remain to be studied.

**Key Words: *Lantana camara*, Crude extract, *Spodoptera litura*.**

## INTRODUCTION

*Lantana camara* L. is cultivated in Thailand as an ornamental plant. It has been regarded as weed in some places because of its rapid growth. It is used in folk remedies for treatment of cancers, fevers, influenza, cold, joint diseases and ulcers[1]. *L. camara* is also used for its antimutagenic and antibacterial actions[2,3]. Essential oil of *L. camara* inhibited growth of *Pseudomonas aeruginosa*, *Aspergillus niger*, *Fusarium solani*, *Candida albican*, *Staphylococcus typhi*, *Bacillus subtilis* and *Bacillus aureus*[4-8]. Moreover, *L. camara* also has insecticidal properties to control several pests such as *Sitophilus zeamais*[9], diamondback moth[10] and *Crocidolomia binotalis*[11]. Therefore, this study aimed to investigate *L. camara* flower crude extracts for pest controls. *Spodoptera litura* was a target pest because it attacks several economic crops worldwide and this species has rapidly developed resistance to many insecticides. *S. litura* is a common pest of agriculture including vegetables, field crops and fruit trees. The life cycle from egg, larva, pupa and adult last for 3-4, 10-14, 7-10 and 7-10 days, respectively.

## EXPERIMENTAL

Fresh flowers of *L. camara* were collected from the Faculty of Liberal Arts and Science, Kasetsart University, Kamphaeng Saen, Nakhonpathom, Thailand, then washed and oven-dried at 50 ºC until they were brittle. The oven-dried flowers were grounded to fine powders. Flower powder samples (20 g) were sequentially extracted with 400 mL of *n*-hexane by soxhlet apparatus for 8 h. Extracts were placed in a rotary evaporator then evaporated until dryness to remove the solvent. The crude *L. camara* was further dissolved in 400 mL of dichloromethane, extracted and evaporated by vacuum rotary evaporator. The crude product was further extracted with 400 mL methanol and evaporated to dryness. The dry crude extracts from each solvent were kept at 4 ºC until they were later dissolved in ethanol to prepare the concentrations of extract used for testing.

**Test insect:** The larvae of *S. litura* (common cutworm) were obtained from Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand. They were reared on artificial diet containing green bean, in room temperature at 25-26 ºC until they became to second or third instar larvae.

**Contact toxicity:** Ten of 2nd instar larvae were tested for contact toxicity by applying 2 µL of each crude flower extract (1, 4, 7 and 10 % w/v) and ethanol on the third thorax of *S. litura*. After crude extracts were dry, the larvae were placed on artificial diets. The dead larvae were counted every day for 7 days[12]. The contact toxicity experiment was arranged by completely randomized design (CRD). Each treatment was conducted with five replicates.

**Oral toxicity (no choice test):** Oral toxicity was tested by the leaf disk method. Chinese kale leaves were cut into circle disks of 2 cm diameter by a cork borer then the disks were treated on their upper surface with 30 µL of *n*-hexane, dichloromethane and methanol crude extracts (1, 4, 7 and

10 % w/v) or ethanol (control). After dryness, each leaf disk was placed on a moist filter paper in a Petri dish. The larvae of 2nd instar, fasted for 6 h, were placed in the Petri dish. After each larva consumed all of the leaf disks, it was returned to its artificial diet of green bean. The dead larvae were counted every day for 7 days. Ten larvae were tested in each treatment and each treatment was conducted in five replicates.

**Repellent and antifeedant activity:** Repellent and antifeedant activity was determined in *S. litura* larve (third instar) that had access to treated and untreated leaves. Leaf disks (0.6 $cm^2$) were cut from Chinese kale leaves with a cork borer and used as substrates for testing extracts and controls. The leaf disks were treated on their upper surface with 20 µL of test solutions of plant extracts at concentrations of 1, 4, 7 or 10 % w/v. Control leaf disks were treated with the same volume of 95 % ethanol. When the solvent was evaporated, two treated and two control leaf disks were placed on a moist filter paper in a Petri dish and presented to two larva. Results were taken when approximately 50 % of the total area of the control disks in each Petri dish was 0.3 $cm^2$. The scoring system rated the feeding in the dishes as R, A, a and –. R: repellent activity, feeding inhibition without tasting treated leaf disks. A: strong antifeedant activity, less than 5 % of the total area of treated leaf disks in each Petri dish consumed. a: antifeedant activity, 5-20 % of the total area of treated leaf disks in each Petri dish consumed. -: inactive, more than 20 % of the total area of treated leaf disks in each Petri dish consumed[13]. The experiment was arranged by completely randomized design (CRD). Each experiment was conducted with five replicates.

**Data analysis:** The per cent mortality was calculated in the uniform population of larvae using Abbott's formula, which considers the natural mortality of untreated controls in the denominator[14]. Probit analysis was used to estimate $LC_{50}$ value and ANOVA was computed using the SPSS version 17.0 software package. The means of each treatment were compared by the one way-ANOVA and Duncan's multiple range test (DMRT) with a predetermined significance level $\alpha < 0.05$.

## RESULTS AND DISCUSSION

Extraction of dry flowers of *L. camara* using Soxhlet produced *n*-hexane fraction 3.16 % w/w, dichloromethane extract 1.08 % w/w and methanol extract 21.07 % w/w.

**Contact toxicity:** Topical applications of crude extracts of *n*-hexane, dichloromethane and methanol were tested for insecticidal activity against *S. litura*. The crude flower extracts (10 % w/v) were applied on the third thorax of larvae (second instar), then the larvae were introduced to their artificial diet. The contact toxic effects of dichloromethane and *n*-hexane extracts were significantly higher than those of methanol and control (Table-1) and increased between 1 and 7 days of exposure. The crude dichloromethane extract caused 32 % of the larvae to die in the first day and 56 % (55.1 % corrected mortality) to die within 7 days. The *n*-hexane extract caused 34 % (32.65 % corrected mortality) of the larvae to die in 7 days. Therefore, the crude extracts from flowers of *L. camara* had an acute contact toxicity which was similar to the results of Morallo-Rejesus[10] which showed the essential oil of *L. camara* flowers exhibited contact toxicity to *Plutella xylostella* with $LD_{50}$ equal to 5.498 mg/g body weight.

TABLE-1
PER CENT MORTALLITY OF *S. litura* AFTER CONTACT WITH DIFFERENT CRUDE EXTRACTS FROM THE FLOWERS OF *L. litura* AT 10 % w/v

| Treatment | Mortality (%) | | Total mortality (%) | Corrected mortality (Abbott's formula) |
|---|---|---|---|---|
| | 1 day | Day 2-7 | In 7 days | |
| *n*-Hexane | 26 | 8 | $34^b$ | $32.65^b$ |
| Dichloromethane | 32 | 24 | $56^c$ | $55.1^c$ |
| Methanol | 2 | 0 | $2^a$ | $0^a$ |
| Control | 0 | 2 | $2^a$ | – |

$^{a,b,c}$:Means within rows followed by the different letters were significantly different by DMRT ($p < 0.05$).

The mortality of the larvae increased as concentrations of dichloromethane extracts increased (Fig. 1), providing an $LC_{50}$ of 9.453 % w/v at 7 days. However, the mortality of larvae treated with *n*-hexane extract did not increase as the concentration increased from 7-10 % w/v. This may be related to the limited permeation of the nonpolar crude extract through the cuticle of the larvae.

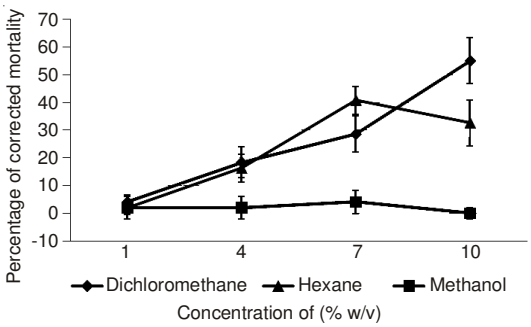


Fig. 1. Mean per cent mortality of *S. litura*'s 7 days after contact application of different concentrations of *n*-hexane, dichloromethane and methanol flower crude extracts from *L. camara*

**Oral toxicity:** Crude extracts from flowers of *L. camara* were applied on Chinese kale leaf discs and fed to the larvae. No toxicity was observed after one day of feeding but some toxicity was noted by 7 days. These mortalities were less than 20 % and no treatment showed significant differences from the controls (Table-2). Although results indicated that there was no acute oral toxicity, it is possible that longer exposures to *L. camara* may deter larval development or inhibit growth. For example, Wheeler *et al.*[15] tested the biological activity of a crude methanolic extract of *Trichilia americana* with *S. litura* and reported that the crude extract reduced growth, consumption and utilization of ingested and digested food when fed to larvae.

**Leaf disk choice test:** After the two larvae were introduced into the Petri dish that contained two control and two treated leaf disks, the areas of leaf disks they consumed were measured to assess repellent or antifeedant properties. The dichloromethane and *n*-hexane extracts showed antifeedant property to *S. litura* (Table-3). The crude dichloromethane extracts inhibited feeding of larvae in concentrations as low as 1 % w/v but the *n*-hexane extracts had to be 4 % w/v before having an effect. However, both extracts contained chemicals which deter feeding. No antifeeding and repellent effects were observed with methanol extracts. Other reports demonstrate

TABLE-2
PER CENT MORTALITY OF *S. litura's* AFTER ORAL ADMINISTRATION OF DIFFERENT CRUDE EXTRACTS FROM *L. litura* FLOWERS AT 10 % w/v

| Treatment | Mortality (%) | | Total Mortality 7 day (%) |
|---|---|---|---|
| | 1 day | Day 2-7 | |
| *n*-Hexane | 0 | 10 | 10* |
| Dichloromethane | 0 | 16 | 16* |
| Methanol | 0 | 4 | 4* |
| Control | 0 | 6 | 6* |

*Means within rows followed by the same letters were not significantly different by DMRT ($p > 0.05$).

TABLE-3
FEEDING DETERRENCE AND REPELLENT BIOASSAYS OF CRUDE EXTRACTS FROM *L. camara* FLOWERS ON *S. litura*

| Treatment | Consumed areas and activities at the following concentrations of crude extract | | | |
|---|---|---|---|---|
| | 10 (%) | 7 (%) | 4 (%) | 1 (%) |
| Methanol | 28.17 (–)* | 42.83 (–) | 38.83 (–) | 32.83 (–) |
| Dichloromethane | 11.67 (a) | 5.74 (a) | 5.33 (a) | 11.07 (a) |
| *n*-Hexane | 15.52 (a) | 17.87 (a) | 14.59 (a) | 49.34 (–) |

*The feeding preference of third instar larvae of *S. litura* was observed in a leaf disk choice bioassay in order to assess repellent or antifeedant properties. The assays were conducted in five replicates. To avoid a non-choice situation, results were taken when approximately 50 % of the total area of control disks in each Petri dish was eaten. The scoring system used R, A, a, and – for rating. R: repellent activity, feeding inhibition without tasting treated leaf disks. A: strong antifeedant activity, less than 5 % of the total area of treated leaf disks in each Petri dish was consumed. a: antifeedant activity, 5-20 % of the total area of treated leaf disks in each Petri dish was consumed. -: inactive, more than 20 % of the total area of treated leaf disks in each Petri dish was consumed[13].

effects of plant extracts on insect feeding behaviour. For example, aqueous extracts of *L. camara* reduced feeding of the *P. xylostella* larvae about 80-fold compared to control (using the no-choice test and wet method)[10]. Dong *et al.*[16] reported that crude lantadene, extracted from *L. camara* leaves, showed antifeeding activity on *S. litura* using the no choice test. They reported that crude lantadene at 1.6 mg/mL had antifeeding effects on 1st instar *S. litura* larvae with an antifeeding rate of 33.1 %[16]. Brem *et al.*[13] tested feeding deterrence effects of *Stemona collinsae* and -*Stemona tuberose* to fifth instar larvae of *S. littoralis* (the same family as *S. litura*). The crude leaves and roots extracts of *Stemona collinsae* showed strong feeding inhibitory properties against *S. littoralis* in a leaf disk choice test, exhibiting feeding inhibition at concentrations as low as 10 μg/cm$^2$.

*L. camara* extracts have previously been reported to exhibit insecticidal properties to control several pests, including *Sitophilus zeamais*[9], diamondback moth[10] and *Crocidolomia binotalis*[11]. This research reports that crude flower extracts of *L. camara* showed toxic effects on *S. litura* as a contact poison and as an antifeedant. However, use of plant extracts alone for control of pest insects may be made more beneficial when combined with other insecticides. For example, Facknath[11] reported that efficacies of three plant extracts (*Azadirachta indica*, *Ayapana triplinervis* and *Lantana camara*) were enhanced by combination with *Bacillus thuringiensis* (Bt) when used to control *P. xylostella* and *Crocidolomia binotalis* infestations of field cabbages. Results showed that the combination treatments of *Bacillus thuringiensis* with a botanical were significantly more effective in reducing the rate of increase in *P. xylostella* and *Crocidolomia binotalis* populations. Facknath[11] suggested that the botanicals had enhancing influence on the *Bacillus thuringiensis* and that the combination of any plant extract with *Bacillus thuringiensis* would be more efficient than the individual botanical or *Bacillus thuringiensis* treatment alone. Both botanicals and *Bacillus thuringiensis* are environmental-friendly, rapidly degraded in sunlight. The use of botanicals and *Bacillus thuringiensis* together could constitute an effective and environmentally safe integrated pest management program.

## Conclusion

*L. camara* flower crude extracts obtained by Soxhlet extraction showed toxic effects on *S. litura*. The dichloromethane and *n*-hexane crude extracts from *L. camara* flowers exhibited contact toxicity and kept the second instar of *S. litura* from feeding. The acute contact toxicity with the median lethal concentration ($LC_{50}$) of dichloromethane extract was 9.453 % w/v. The dichloromethane and *n*-hexane extracts also showed antifeedant activity on the third instar of *S. litura* without causing mortality after ingestion. However, these initial experiments show insecticidal success of these botanical extracts, suggesting a need for longer term studies and/or studies using different extraction or purification procedures.

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