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Screening of Thai Local Plant Extracts for their Insecticidal Effectiveness and the Effect of its Active Compound on Diamondback Moth Larvae

Kritchaya Issakul[ab], Elke Pawelzik[a], Araya Jatisatienr[b], Chaiwat Jatisatienr[b], Suchada Vearasilp[c]

a Georg-August-Universität Göttingen, Department of Crop Science, Germany
b Chiang Mai University, Department of Biology, Thailand
c Chiang Mai University, Department of Agronomy, Thailand

Introduction

With the rising concern for environmental safety there has been a renewed interest in the use of naturally occurring substances as pesticides, including plant bioactive compounds. Many naturally insecticides have active control agents for a variety of insect pests. Diamondback moth is one of the most damaged insect pests of *Brassica* crops and there are also some reports on the resistance of the diamondback moth against many synthetic insecticides. Therefore, searching for new botanical insecticides for controlling the diamondback moth is still essential. In present work, some Thai native plant extracts were investigated for their insecticidal property. Moreover, the bioactive compound of the selected plant extract was purified, identified and confirmed for its insecticidal effectiveness against diamondback moth.

Material and Methods

Plant selection

Some plants with known insecticidal properties were selected from secondary data i.e. some reports in the literature or some bioethnological knowledge by farmers. Nine plant species showing insecticidal activities were selected. These species were *Acorus calamus*, *Eugenia caryophyllus*, *Mammea siamensis* and 6 species of *Stemona* (*S. curtisii*, S. *tuberosa*, *S. burkillii*, *S. kerrii*, *Stemona* unknown 1 and *Stemona* unknown 2).

Brine shrimp lethality test method (BST) (Teng, 1993) was used as a general screening bioassay to select the most effective plants from all the plants tested. The toxic activities of all plant extracts tested on brine shrimp (*Artemia salina* Leach) were determined. The 48 hours old brine shrimp were tested with nine plant extracts. All the stock solutions of plant crude extracts were dissolved in artificial sea water at different concentrations. Each test solution was prepared in triplicate. Ten brine shrimps were dropped into each test solutions in test tubes. The mortality of brine shrimp was observed under a stereomicroscope at 24 hours after application. The dead nauplii were used to determine the LC_{50}. The data were analyzed by probit analysis, SPSS for Windows.

Extraction, purification and identification of bioactive compound from selected plant

The most effective plant extract was selected for the purification of its bioactive compound. This plant extract was purified using column and preparative thin layer chromatographic methods. The brine shrimp lethality test (BST) was used for selecting the active compounds isolated from each chromatographic step again.

The most effective compound was elucidated for its structure by IR, NMR and MS techniques. IR spectra were obtained on a Nicolet AVATAR 300 FTIR spectrophotometer. ^{1}H NMR spectra were recorded on Varian Mercury 300 and Varian Unity 500 spectrometers. High resolution EIMS were recorded on a Fison/VG Autospec-TOF-oa mass spectrometer (70 eV) and polyethyleneglycol (PEG) as an internal reference.

Determination of insecticidal properties of surangin B

The insecticidal properties of surangin B were investigated against diamondback moths larvae (*Plutella xylostella* Linn.) in comparison with the commercial insecticide methomyl and untreated sample (control).

Anti-feedant activity tests

The anti-feedant activity of active substance was assayed by the leaf dipping method (Busvine, 1980). Petioles were wrapped with wet cotton wool and covered by aluminum foil to maintain leaf moisture. Three leaves per dose were dipped in the test solution for 30 seconds and kept at room temperature to evaporate the solvents. Surangin B and methomyl were dissolved in acetone to reach five levels of the final concentrations. Ten diamondback moths were randomly selected and fed on the tested leaves placed in Petri dish. The area of damaged leaf was measured in a digital format 24 hours after application by Image 4.0.2 for Windows. (O' Neal *et al*., 2002).

Contact activity test

The contact activity of active substance was investigated using a topical application method (Busvine, 1980). Surangin B and methomyl were dissolved in acetone to reach five levels of the final concentrations. One microliter of the test substance was topically applied to the dorsal thorax of the 3rd instar larvae of the diamondback moth. Dead larvae were counted 24 hours after application. All treatments were done in triplicate, and 10 larvae were used for each replication. The percent mortality was determined 24 hours after application. Probit analysis was used for the calculation of LC_{50} values.

Results and Discussion

Plant selection

The toxic activity of nine plant extracts was investigated by the brine shrimp lethality test. The results indicated that *Mammea siamensis* had the highest efficiency against brine shrimp with the LC_{50} value of 0.072 mg L^{-1}, followed by *Stemona curtisii, Eugenia caryophyllus, Stemona kerrii, Stemona* unknown 2, *Stemona* unknown 1, *Stemona burkillii, Stemona tuberosa* and *Acorus calamus,* respectively (Table 1).

Table 1 The 24 hour LC_{50} values of plant extracts on brine shrimp

Plant extracts	**24 hr LC_{50} values (mg L^{-1})**
Acorus calamus	295.45
Eugenia caryophyllus	58.54
Mammea siamensis	0.072
Stemona burkillii	240.00
Stemona curtisii	50.05
Stemona kerrii	59.18
Stemona tuberosa	253.27
Stemona unknown 1	75.75
Stemona unknown 2	59.70

Extraction, purification and identification of bioactive compound from selected plant

Due to its highest efficiency against brine shrimp, *Mammea siamensis* was selected for the purification and identification of the active insecticidal substances by chromatography and also

by using BST-guided fractionation. The toxic activity of all fractions of mammea compounds was determined by 24 hour LC_{50} of brine shrimp. Fraction 5.1 had the highest toxic activity on brine shrimp with a LC_{50} value of 0.014 mg L^{-1}. It was separated by chromatographic method as the following;

Five gram of mammea ethanolic crude extract was partitioned by hexane and methanol. Four grams of hexane fraction was column chromatographed using 500 mL GF_{254} flash silica gel (40-63 μm) by gradient elution [C_6H_6-EtOAc, (100:0 to 0:100)]. A total of 5,000 mL of eluent was collected in 20 mL test tubes. TLC analysis was performed on aluminium-sheet 60 GF_{254} silica gel and bands were detected by UV light at λ 254 nm. On the basis of TLC analysis, these fractions were pooled to give five fractions. The further separation of fraction 5 by column chromatography with a gradient elution [C_7H_8-EtOAc, (80:20 to 0:100)] and then by preparative TLC (C_6H_6-EtOAc, 90:10) gave fraction 5.1 (225 mg).

A potential bioactive compound (fraction 5.1) was isolated as a yellow brown gum. High resolution mass spectrometry (HRMS) was used to determine the molecular structure and the HRMS result (EI +ve, *m/z* [M]+, 498.2625, calculated 498.2617) indicated that this compound has the molecular formula $C_{29}H_{38}O_7$ which was supported by a previous study on *Mammea* by Mungkornasawakul (2004). It could be assumed that this bioactive compound might be surangin B. Infrared spectroscopy (IR) was also used to determine the functional groups of this compound and the IR spectrum exhibited characteristic absorption bands for a hydroxyl (br, 3400 cm^{-1}), a δ-lactone (1743 cm^{-1}) and a H-bonded acyl group (1594 cm^{-1}), an aromatic (1560 cm^{-1}) and C-O stretch for the acetate group (1231 cm^{-1}) which was also related to the structure of surangin B reported by Mungkornasawakul (2004). The confirmation of the structure was done by NMR spectroscopy. The ^1H NMR spectroscopic data of the bioactive compound was compared with published results (Joshi *et al*., 1969) for surangin B. The IR, MS and NMR analysis indicated that the bioactive compound was surangin B which belongs to a coumarin group.

Determination of insecticidal properties of surangin B

Anti-feedant activity test

Surangin B isolated from *M. siamensis* showed very high anti-feedant toxicity to the 3rd instar larvae of *Plutella xylostella* L. (diamondback moth) with a very low percentage of damaged leaf area of 0.83 and 0.14% at the concentrations of 0.5 and 1.0 g L^{-1}, respectively. In this study, methomyl used as conventional insecticide was compared with surangin B. It had a lower toxicity than surangin B, the percentage of damaged leaf area was 3.19% at the concentration of 0.5 g L^{-1} and 1.65% at the concentration of 1.0 g L^{-1} (Table 2).

Table 2 Anti-feedant activity of surangin B and methomyl on the 3rd instar larvae of *P. xylostella*

Treatments	**Concentrations (g L^{-1})**	**Percent of leaf area damaged (Mean ± SD)***
Surangin B	0.5	0.83 ± 0.21 **a**
	1.0	0.14 ± 0.05 **a**
Methomyl	0.5	3.19 ± 0.59 **ab**
	1.0	1.65 ± 0.27 **ab**
Control (solvent)	-	7.64 ± 2.52 **b**

Contact activity test

On the basis of LC_{50} values, the 3rd instar larvae of *P. xylostella* were more susceptible to surangin B than to methomyl with LC_{50} values of 0.07 and 0.51 g L^{-1}, respectively (Table 3).

Table 3 Contact activity of surangin B and methomyl on the 3rd instar larvae of *P. xylostella*

Treatments	24 hrs LC_{50} values (g L^{-1})
Surangin B	0.07
Methomyl	0.51

On the basis of both anti-feedant and contact activity. It can be concluded that surangin B expressed stronger toxicity to diamondback moths than methomyl. Zheng *et al.* (1998) proposed that mitochondria blockade accompanied by presynaptic release of neurotransmitters and loss of postsynaptic sensitivity were possibly important mechanisms contributing to paralysis in insects dosed with surangin B. These results indicated that the mitochondrial blockade leading to bio-energetic failure in muscles and nerves was a major mechanism in the development of paralysis in insects exposed to surangin B.

Conclusions and Outlook

Mammea siamensis indicated the highest efficiency against brine shrimp. The ethanolic crude extract of *M. siamensis* was examined for its bioactive compound by chromatographic techniques. Surangin B was identified as the bioactive compound by spectroscopic methods (IR, NMR and MS). It expressed very strong contact and anti-feedant activities on diamondback moth larvae. In conclusion, *M. siamensis* demonstrated enormously satisfying results as a new botanical insecticide. However, further studies on its effects on agricultural products and also on ecosystems need to be studied whether this plant extract could be employed as an alternatively useful natural insecticide.

References

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