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Full Length Research Paper

Potential of *Mammea siamensis* as a botanical insecticide: Its efficiency on diamondback moth and side effects on non-target organisms

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Nine species of local Thai medicinal plant extracts with known insecticidal properties that is, *Acorus calamus, Eugenia caryophyllus*, *Mammea siamensis* and 6 species of *Stemona* (*Stemona curtisii*, Stemona *tuberosa*, *Stemona burkillii*, *Stemona kerrii*, *Stemona* unknown 1 and *Stemona* unknown 2) were screened for the highest insecticidal activity by the brine shrimp lethality test (BST). *M. siamensis* showed a very strong toxic effect on brine shrimp with the lowest 24 h LC_{50} value of 0.072 µgml^{-1}. The purification of its active compound was conducted using chromatographic methods and the BST to select the most effective fraction. The spectroscopic methods were used for the identification of the active compound. Surangin B was finally identified as the active compound. Its insecticidal effectiveness on the 3rd instar larvae of diamondback moth was investigated by topical application and leaf dipping methods in comparison with methomyl. The results indicated that surangin B demonstrated high in both contact and anti-feedant activities than methomyl. According to its side effects on non-target organisms, *M. siamensis* exhibited no negative impact on earthworm and honeybee. In contrast, it showed a higher toxicity on fish than methomyl. From the results it can be concluded, that *M. siamensis* might be one of the natural insecticides for the diamondback moth management.

Key words: *Plutella xylostella* Linn., botanical insecticide, surangin B, bioactive compound.

INTRODUCTION

Pesticides are widely used in protecting agricultural crops from different pests. On the other hand, the hazardous effect of their residues to human health and environment should be concerned. 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. Soejarto and Farnsworth (1989) estimated that out of the 250,000 species of flowering plants, only 5,000 species had been thoroughly investigated according to the natural product alert (NAPRALERT) database, leaving 98% of these species with potential for phytochemical discovery. Approximately 2,500 plants in 247 families had some toxic properties against insects (Heal et al., 1950).

Diamondback moth (*Plutella xylostella* Linn.) 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 (Noppun et al., 1983; Agerbirk et al., 2003; Li et al., 2006; Endersby et al., 2008). Therefore, searching for new botanical insecticides for controlling the diamondback

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moth is still essential. Similar to conventional pesticides, the botanical insecticides may cause sometimes negative side effects on non-target organisms. For example, pyrethrum is easily destroyed by light and air but it is toxic to fish and honeybees under laboratory testing (Perry et al., 1998). Neem oil is also toxic to several fish species. The seeds, whole fruits, or leaves of *Melia azedarach* contain compounds which are highly toxic to mammals (Schmutterer, 1995). In present work, some Thai medicinal plant extracts were investigated for their insecticidal property. The bioactive compound of the selected plant extract was purified, identified and confirmed for its insecticidal effectiveness against diamondback moth. Moreover, the adverse effects of the selected plant extract were also investigated on some non-target organisms.

MATERIALS AND METHODS

Plant selection

Nine plant species showing insecticidal activities were selected from secondary data. These species were collected from different locations in Thailand. The rhizomes of *Acorus calamus*, *Stemona burkillii*, *Stemona kerrii* and *Stemona tuberosa* were collected at Mae Hea, Amphur Muang, Chiang Mai. The bud flowers of *Eugenia carryophyllus* were purchased from Lanna Herbal Shop, Waroros Market, Muang District, Chiang Mai Province. The seeds of *Mammea siamensis* were collected at Chiang Mai University, Muang District, Chiang Mai Province. The rhizomes of *Stemona curtisii* were collected at Kaunmao, Rasda District, in the North of Trang Province. Living rhizomes plants of an unidentified *Stemona* species (*Stemona* sp1.) were bought at the Tung Kwien market, Lampang Province. The intact plants of an unidentified *Stemona* species (*Stemona* sp2.) were bought at the market in Phra Si Rattana Mahathat Temple, Amphur Muang, Phitsanulok Province. All plant samples were deposited at the Herbarium of the Department of Biology, Chiang Mai University. All plant materials were washed and dried by circulating dry air in an oven at 50°C and then chopped into small pieces. The materials were then soaked in 95% ethanol (Lab-scan, Ireland) and placed in an ultrasonic bath for 45 min, filtered by filter paper No. 1, 90 mm diameter and concentrated by evaporation under reduced pressure with a rotary evaporator at 50°C.

All the crude extracts were dissolved in 95% ethanol and distilled water (1:1) to obtain the total concentration of 10% w/v of stock solutions. Brine shrimp lethality test method (BST) (Teng, 1993) was used as a general screening bioassay to select the most effective plants. The 48 h old brine shrimp (*Artemia salina* Leach) 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 h 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 BST was used for selecting the active compounds isolated from each chromatographic step. 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 active compound

The insecticidal properties of active compound (surangin B) were investigated against diamondback moths larvae (*P. xylostella* Linn.) in comparison with the commercial insecticide methomyl (du Pont, Taiwan) and untreated sample (control).

Anti-feedant activity tests

The anti-feedant activity of active substance was assayed according to method of Ling et al. (2008). 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 s and kept at room temperature to evaporate the solvents. Surangin B (bioactive compound) and methomyl were dissolved in acetone (J.T. Baker, USA) 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 h 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 described by Hashim and Devi (2003). Surangin B and methomyl were dissolved in acetone to reach five levels of the final concentrations. One micro liter 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 h after application. All treatments were done in triplicate, and 10 larvae were used for each replication. The percent mortality was determined 24 h after application. Probit analysis was used for the calculation of LC_{50} values.

Toxicity of mammea insecticide on some living organisms

Toxicity of mammea crude extract on non-target insects (honeybee), earthworm and fish was investigated in the laboratory in comparison with methomyl insecticide. The toxic level of methomyl and mammea crude extract on the studied organisms was determined as LC_{50} value.

Toxicity of *M. siamensis* on Thai species of earthworm *(Pheretima peguana)*

The contact toxicity test was conducted to determine an acute toxicity of mammea crude extract in comparison with methomyl using filter paper contact test according to Robidoux et al. (1999). Flat-bottomed glass vials approximately 7 cm in length and 2.5 cm in diameter were used. Their sides are lined with 6.0 x 6.5 cm (39 cm^2) filter paper. The test solutions were pipetted onto the filter paper in each vial to reach the final concentrations of

Table 1. The 24 h LC_{50} values of plant crude extracts on brine shrimp.

Plant extracts	24 h LC_{50} values (μgml^{-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 sp.1	75.75
Stemona sp. 2	59.70

0.0001 to 1.0 mg $(cm^2)^{-1}$. A single worm was placed on each vial and sealed with a cap with a small ventilation hole. Ten replications were carried out for each concentration. The vials were kept in total darkness and the evaluations were made 24 h after treatment. The same procedure was conducted to determine the toxicity of both mammea crude extract and methomyl. LC_{50} values were calculated using probit analysis.

Toxicity of *M. siamensis* on honeybee (*Apis mellifera* L.)

Acute contact toxicity (topical application and filter paper method) was conducted to determine the toxicity of mammea crude extract and methomyl on honeybee. Honeybees were obtained from an organic commercial honeybee culture, Chiang Mai, Thailand. Test bees were obtained directly from hives to the cages of 13.5 x 19.8 x 7.0 cm with a 7.8 cm diameter nylon hole on the top of the cover. The cages were kept in an incubator at 29.7 ± 2°C, 67 ± 5% relative humidity and fed with honey (honey : water = 9:1) for 3 h before application.

Topical application method

The acute contact toxicity of mammea crude extract and methomyl on honeybee was investigated according the OECD guideline (Organization for Economic Co-operation and Development, 1998). Mammea crude extract and methomyl were dissolved in ethanol with 5 different concentration levels. Test bees were immobilized with CO_2 for 10 seconds and 10 bees were immediately placed in the holding cage of 5.6 x 6.8 x 2.7 cm with a 3.6 cm diameter nylon hole on the top of the cover. One micro liter of the test substances was applied topically to the ventral thorax of the bees with the micro-applicator. Two milliliters of 90% honey (honey : water = 9 : 1) was deposited on the cotton wool and then placed on the nylon hole for feeding. Five replications were performed for each concentration. Dead honeybees were counted and recorded 24 h after application. Probit analysis was used for the calculation of LC_{50} values.

Filter paper method

Filter paper method was conducted to determine the contact activity of mammea crude extract with some modifications of the procedure described by Liu and Ho (1999). Five different dosage levels of mammea crude extract and methomyl were performed. Whatman filter paper No.1 was cut to 5.6 x 6.8 cm and placed on the bottom of holding cages of 5.6 x 6.8 x 2.7 cm with a 3.6 cm diameter nylon hole on the top of the cover. Two milliliters of 90% honey (honey : water = 9 : 1) was deposited on cotton wool and then placed on the nylon hole for feeding. One milliliter of the test substances was applied on the paper and left until dryness. Ten immobilized bees were placed at the bottom of the holding cage. The dead honeybees were counted and recorded 24 h after application. The probit analysis was used for the calculation of LC_{50} values.

Toxicity of *M. siamensis* on the fingerlings of *tilapia* (*Oreochromis niloticus*)

The toxicity of mammea crude extract on tilapia was determined according to the method of the OECD guideline (Organization for Economic Co-operation and Development, 1992). Fingerlings (35 to 40 mm length) of *tilapia* were obtained from the Faculty of Fisheries Technology and Aquatic Resources, Maejo University, Chiang Mai, Thailand. The fishes were allowed to acclimatize to the laboratory conditions in glass aquaria filled with continuously aerated aged tap water under natural photoperiod for 1 week. Half of the water in the aquarium was replaced with aged tap water every 3 days. The experiments were designed to expose the fish to mammea crude extract and methomyl during the experimental period with 3 replications (20 fishes/replicate) at each dosage level.

Methomyl and mammea crude extract were prepared freshly by dissolving both substances with aged tape water to reach 7 different concentrations in a glass aquarium (30 x 60 x 38 cm) which containing 44 L of the test water. The 7 different concentrations of methomyl were also prepared. Fish mortality was recorded 96 h after application. The percent mortality data was calculated for the 96 h LC_{50} values by probit analysis.

RESULTS AND DISCUSION

Plant selection

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

Extraction, purification and identification of bioactive compound from selected plant

Due to its highest efficiency against brine shrimp, *M. 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 h LC_{50} of brine shrimp (Table 2). Fraction 5.1 had the highest toxic activity on brine shrimp with a LC_{50} value of 0.014 μgml^{-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

Table 2. The LC_{50} values of *Mammea* fractions on brine shrimp.

***Mammea* fractions**				LC_{50} (μgml^{-1})
Ethanolic crude				0.072
Hexane crude				0.056
	Fraction 1			0.226
	Fraction 2			0.443
	Fraction 3			0.042
	Fraction 4			0.032
	Fraction 5			0.288
		Fraction 4.1		0.289
		Fraction 4.2		0.211
			Fraction 4.2.1	0.199
			Fraction 4.2.2	0.400
			Fraction 4.2.3	0.083
			Fraction 4.2.4	0.163
		Fraction 4.3		0.141
			Fraction 4.3.1	0.158
			Fraction 4.3.2	0.123
	Fraction 5			0.288
		Fraction 5.1		0.014

ml GF_{254} flash silica gel (40 to 63 µm) by gradient elution [C_6H_6-EtOAc, (100:0 to 0:100)]. A total of 5,000 m| of eluent was collected in 20 ml test tubes. Thin layer chromatography (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 (Table 3). 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 active compound

Surangin B isolated from *M. siamensis* showed very high anti-feedant toxicity to the 3rd instar larvae of *P. 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 $mgml^{-1}$, 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 mg ml^{-1} and 1.65% at the concentration of 1.0 mg ml^{-1} (Table 4). Considering to the contact activity test, 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 $mgml^{-1}$, respectively (Table 5).

Toxicity of *M. siamensis* on some living organisms

Toxicity of M. siamensis on Thai species of earthworm (Pheretima peguana)

Methomyl was found as a very toxic substance to the worms within 72 h with LC_{50} values of 0.04 µg $(cm^2)^{-1}$ while mammea crude extract was less toxic with the LC_{50} value of 1.92 µg $(cm^2)^{-1}$ (Table 6).

Table 3. Comparison of the ^{1}H NMR (500 MHz) spectroscopic data of the active substance (experimental) and the ^{1}H NMR (300 MHz) spectroscopic data surangin B in $CDCl_3$ solution (Joshi et al., 1969).

Position	Experimental δ_H [mult., *J* (Hz)]	Reference δ_H [mult., *J* (Hz)]
3	6.28 (d, 1.2)	6.2 (br s)
OH-5	7.25 (s)	7.2 (s)
OH-7	14.7 (s)	14.8 (s)
9	6.41 (t, 8.1)	6.45 (dd)
10	1.6-1.4 (m)	1.6-1.4 (m)
11	1.08 (t, 7.7)	1.0 (t)
1’	3.50 (d, 6.6)	3.4 (d)
2’	5.25 (t, 6.3)	5.2 (t)
4’	2.12 (d, 2.1)	2.1 (d)
5’	2.12 (d, 2.1)	2.1 (d)
6’	5.06 (br s)	5.05 (m)
7’	1.68 (s)	1.65 (s)
8’	1.60 (s)	1.58 (s)
2’’	3.80 (sept, 2.1)	3.8 (q)
3’’	1.6-1.4 (m)	1.8-1.4 (m)
4’’	1.08 (t, 7.2)	1.0 (t)
5’’	1.25 (d, 6.6)	1.25 (d)
OAc	2.18 (s)	2.2 (s)

Table 4. Anti-feedant activity of surangin B and methomyl on the 3rd instar larvae of *P. xylostella*.

Treatments	Concentrations (mgml^{-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

* The different letters indicate the statistically significant difference.

Toxicity of *M. siamensis* on honeybee *(Apis mellifera L.)*

The honeybees appeared to be more susceptible to methomyl than mammea crude extract in both topical application and filter paper method with very low LC_{50} values of 3.05 µg bee^{-1} and 0.00053 mg (cm^2)$^{-1}$, respectively. Mammea crude extract was safer for the honeybee with the LC_{50} values for topical application and filter paper method of 106.08 µg bee^{-1} and 3.14 mg (cm^2)$^{-1}$, respectively (Table 7).

Toxicity of M. siamensis on the fingerlings of tilapia (Oreo-chromis nilotieus)

Mammea crude extract was found to have a very strong toxicity on the fingerlings of *tilapia* (*O. niloticus*) with 96 h LC_{50} values of 0.096 mgL^{-1} while methomyl gave a higher LC_{50} value of 4.861 mgL^{-1} (Table 8).

DISCUSSION

The IR, MS and NMR analysis indicated that the bioactive compound of *M. siamensis* was surangin B which belongs to a coumarin group. Four coumarins isolated from the crude seed extract of *M. siamensis* that is surangin B, surangin C, mammea E/BB and mammea E/BC were also reported by Mungkornasawakul (2004). While, Mahidol et al. (2002) found 4 new *Mammea* coumarins isolated from the flowers of *M. siamensis* that is mammea E/BA cyclo D, mammea E/BC cyclo D, mammea E/BD cyclo D, and mammea E/AC cyclo D. The

Table 5. Contact activity of surangin B and methomyl on the 3rd instar larvae of *P. xylostella*.

Treatments	24 h LC_{50} values ($mgml^{-1}$)
Surangin B	0.07
Methomyl	0.51

Table 6. The LC_{50} values of *M. siamensis* and methomyl on the earthworm.

Test substances	72 h LC_{50} values ($\mu g\ (cm^2)^{-1}$)
Mammea siamensis	1.92
Methomyl	0.04

insecticidal properties of *mammea* have been described by many investigators and the active compounds were usually coumarins. Mammein, the insecticidal compound from the seeds of *Mammea americana*, was the first naturally occurring coumarin with a *n*-propyl substituent at C-4 (Morris and Pagan, 1953) whereas mammeisin was isolated from the fruits peels of *M. americana* (Finnegan et al., 1961). Crombie et al. (1972) reported 4-alkyl-5-7-dihydroxy coumarins namely, mammea E/BA and mammea E/BB which also displayed insecticidal activities. The insecticidal activity of various parts of the mammea tree was studied and it was evident in both feeding and contact experiments, that the seed kernel extract was the most effective part against armyworms, melonworms, cockroaches, ants, drywood termites, mosquitoes and their larvae, flies, aphids and the larvae of diamondback moths (Morton et al., 1987). *M. siamensis* had a very high efficiency against the fourth instar larvae of *Aedes aegypti* with a LC_{50} value of 5.9 μgml^{-1} (Promsiri et al., 2006). Moreover, Issakul et al. (2004) investigated insecticidal substances extracted from *M. siamensis* and reported the insecticidal effects of ethanolic crude extract of *M. siamensis* on the eggs of the housefly, *Musca domestica*.

According to anti-feedant and contact activities. 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. Considering the relationships between chemical structure and insecticidal activity, Mungkornasawakul (2004) showed that four isolated coumarins, that is, suragin B, surangin C, mammea E/BB and mammea E/BC, have an acetoxy group on carbon which is connected to the 9 position of surangin B structure. This acetoxy group is found in the most active coumarins, whereas the inactive compound was un-substituted at this position. Crombie et al. (1972) also reported that the 1'-acetoxy-group attached to the 4-alkyl substituent appears to be an important factor in the conferment of insecticidal properties.

According to *M. siamensis* side effects on non-target organisms, it could be indicated that methomyl was 50 times more toxic to Thai species of earthworm (*Pheretima peguana*) than the *M. siamensis*. Lydy and Linck (2003) investigated the toxicity of pesticides on *Eisenia fetida* by filter paper method reported that chlorpyrifos, atrazine and cyanazine expressed the 96 h LC_{50} values of 8.3, 2.9 and 4.9 $\mu g\ (cm^2)^{-1}$, respectively. Therefore, it seemed that the botanical insecticide in this study caused higher toxicity than the studied synthetic pesticides. Nevertheless, the comparison with findings of other authors is of limited validity because of the different genus of earthworm used in this experiment (*P. peguana*) and in that described in the literature (*E. fetida*). Ma and Bodt (1993) determined the effect of chlorpyrifos insecticide on 6 species of earthworm and showed that a large difference of LC_{50} values (more than 9-folds) was found between the most susceptible (*Lumbricus rubellus*) and the resistant (*Eisenia veneta*) genus.

It was obvious that methomyl was more toxic to honeybee on contact test, nearly 35 times for topical application and nearly 6,000 times for filter paper method than the *M. siamensis*. This result was supported by the Environmental Protection Agency's data which specified that methomyl is categorized as highly toxic to bees on an acute contact basis (EPA, 1998). Moreover, Johansen (1977) reported that methomyl is one of insecticides which produced poisoning hazard on honeybee at any time of blooming crops.

Interestingly, the *M. siamensis* was nearly 50 times more toxic than methomyl. However, the WHO (1992) described the toxicity of rotenone, the active compound in the derris extract, on different fish species and for daphnids (water fleas) with a 96 h LC_{50} value of 0.02 to 0.2 mgL^{-1} which indicated that the *M. siamensis* was slightly less toxic than rotenone. Morton (1987) also reported that the extracts of *M. americana* seeds were acutely toxic to fish but 50 to 100 times less potent than derris. Furthermore, Promsiri et al. (2006) also reported that from 112 medicinal plant extracts only mammea crude extract was slightly toxic to guppy fish. Balza et al. (1989) determined the piscicidal activity of mammea crude extract and its purified compound namely proanthocyanidin on *Oreochromis mossambicus* and found that both substances were highly toxic to *tilapia* fingerling with the 24 h LC_{50} values of 0.7 and 0.3 mgL^{-1} respectively. However, it could not be confirmed yet that the strong toxicity of *M. siamensis* on tilapia was achieved by surangin B. Nevertheless, both proantho-cyanidin and surangin B are phenolic compounds and their toxicity on aquatic organisms had been extensively

Table 7. The 24 h LC_{50} values of *M. siamensis* and methomyl on honeybee.

Test substances	24 h LC_{50} values	
	Topical application (µg bee^{-1})	Filter paper method mg $(cm^2)^{-1}$
Mammea siamensis	106.08	3.14
Methomyl	3.05	0.00053

Table 8. The LC_{50} values of *M. siamensis* and methomyl insecticide on the fingerlings of *tilapia.*

Test substances	96 h LC_{50} values (mg L^{-1})
Mammea siamensis	0.096
Methomyl	4.861

reported. Tilapia exposed to phenols showed excess mucous secretion from skin and gill as well as very frequent opercular movements indicating the acute respiratory distress (Saha et al., 1999). Not only tilapia showed a susceptibility to phenolic compounds but also other species of aquatic organisms. DeGraeve et al. (1980) demonstrated that 5 of 8 phenolic compounds tested, produced LC_{50} values for rainbow trout, *Fathead minnows* and *Daphnia publicaria* less than 0.1 mgL^{-1}. *M. siamensis* seemed to be produce a strongly adverse effect on the fish, it might be suggested that the application of *M. siamensis* as botanical insecticide without dilution into the reservoir must be avoided.

Conclusion

M. siamensis indicated the highest efficiency against brine shrimp. The crude ethanolic extract of *M. siamensis* was examined for its bioactive compound by chromatographic techniques. Surangin B was identified as the bioactive compound by spectroscopic methods. It expressed very strong contact and anti-feedant activities on diamondback moth larvae.

Furthermore, *M. siamensis* exhibited no negative impact on earthworm and honeybee. In contrast, *M. siamensis* showed a higher toxicity on fish than methomyl. On the other hand, these are the first results about the application of *M. siamensis* as botanical insecticide and further experiments are necessary to specify the effect on the ecosystem. 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 or not.

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