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Bioactivity of some plant essential oils for seed treatment against pulse beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) on mung bean

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Abstract

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This study was conducted to screen the insecticidal activity of five plant essential oils, subsequently evaluated the seed treatment effect on mung bean seed with the selected plant oil against the pulse beetle, *Callosobruchus maculatus*, under laboratory conditions. Residual contact was conducted to screen insecticidal activity. Insect mortality and LC_{50} were performed after 24, 48 and 72 h of treatment. Clove oil was the most toxic to *C. maculatus* with the lowest LC_{50} values of 16.09, 12.99 and 7.67 µl/ml and achieved the highest mortality after 24, 48 and 72 h of exposure, respectively. Toxicity of plant oils was categorized in a descending order of clove>cinnamon>lengkuas>citronella>kaffir lime. Clove oil was selected to investigate for seed treatment on mung bean seeds to observe the bioactivity of plant oil. In seed dressing application, bioactivity of clove oil was evaluated with five different concentrations of 0.4, 0.8, 1.2, 1.6 and 2 ml/kg on mung bean seeds. Results show 100% mortality of *C. maculatus* adults at the concentration of 2 ml/kg seeds after 5 days of exposure, and mortality percentage of 22.50 ± 4.79 at the lowest concentration level of 0.4 ml/kg seeds after the same exposure time. Furthermore, the five concentrations of clove oil significantly reduced F1 progeny, seed damage, weight loss of mung bean, and increased the inhibition rate. Findings of this study suggest that clove oil can be used as an alternative to synthetic insecticides for integrated pest management against insect pests, especially *C. maculatus*, on mung bean seeds under storage conditions.

Key words: essential oils; bioactivity; *Callosobruchus maculatus*; mung bean

Introduction

Stored product pests are a great challenge in our economy because they infect and contaminate stored agricultural products and animal feed. Stored products are frequently damaged by insect pests and this may account to 5-10% in temperate zones and 20-30% in the tropics (Nakakita, 1998). Mung bean, *Vigna radiata* L. (Wilczek) is seriously infested by pulse beetles *Callosobruchus maculatus* (F.) and *Callosobruchus chinensis* (Coleoptera: Chrysomelidae: Bruchinae) all over the world (Dimetry et al., 2002; Ahmed, 2010; Hafez et al., 2013). *C. maculatus* is one of the most serious pests brought into storage containers with harvested mung bean that can cause total loss of the stored crop in a few months. The estimated post-harvest losses caused by bruchids to the pulses ranged from 30-40% within 6 months and when left unattended losses could be up to 100% (Dongre et al., 1996; Mahendran & Mohan, 2002). In Thailand, two species of bruchid beetles, *C. maculatus* and *C. chinensis,* seriously damage mung bean seeds during storage (Visarathanoonth & Promsatit, 1989).

Mung bean is an important legume crop in South and Southeast Asia because it contains a high content of easily digestible protein, iron and folate (Bains et al., 2003; Weinberger, 2005; Tang et al., 2014). It is also consumed as sprouts, which are an important source of vitamins and minerals (Somta et al., 2007). It is grown widely in South and Southeast Asia countries, mainly India, China, Pakistan, Myanmar, Thailand and Vietnam (Tomooka et al., 2002).

Management of insect pests in many storage systems relies primarily on applying synthetic insecticides. Use of synthetic insecticides is currently the most effective way to prevent the infestation of stored product pests. However, continuous and heavy use of these chemicals has caused adverse effects on non-target organisms, and the development of pesticide resistance in some stored product pests. To solve this problem, many researchers have discovered alternative pest management products derived from plants (Isman, 2006; Adedire et al., 2011). Several plant products have been investigated for insecticidal activities, namely: *Dennetias tripetela*, *Anthozylum zanthoxyloides*, *Eugenia aromatica*, *Nicotiana tabacum*, *Dennetias tripetela* and *Azadirachta indica* (Ogunleye et al., 2004). Plant products are cheap and are easily accessed by farmers and small-scale industries in the form of crude or partially purified extracts. It was indicated that mixing storage pulses and plant products such as leaf, bark, powder or extracted oils reduced the oviposition rate, inhibited the adult emergence of bruchids, and decreased the seed damage rate (Onu & Aliyu, 1995; Shaaya et al., 1997; Keita et al., 2001; Tapondjou et al., 2002).

Citronella oil and cinnamon oil were recorded as the strongest repellent and had the most toxic effects on *C. maculatus* and significantly reduced the oviposition rate and adult emergence after infestation on mung bean seeds (Ratnasekera & Rajapakse, 2009). Lengkuas oil at 0.5% concentration caused 100% mortality of *C. chinensis* adults and total reduction of oviposition on mung bean seeds (Ahmed and Ahmad, 1992). Additionally, clove oil also has biological properties such as antimicrobial activity in food and is a traditional flavoring ingredient (Lee & Shibamoto, 2001; El-Maati et al., 2016). Kaffir lime peel oil also has a strong flavour and antibacterial activity against *Staphylococcus aureus* (Lertsatitthanakorn et al., 2014).

As there is much information on the antibacterial, medicinal and insecticidal activities of these five plant essential oils, they might be very useful for farmers in developing countries to control stored pests such as *C. maculatus*. This investigation was aimed to screen the insecticidal activity of the five plants essential oils by bioassay testing against the beetles and the most effective plant oil was used as a protectant against the *C. maculatus* by seed treatment on mung bean seed under laboratory conditions.

Materials and Methods

Experiment location

The experiment was carried out at Toxicology Laboratory of Pest Management Department, Faculty of Natural Resources, Prince of Songkla University, Hat Yai, Songkhla, Thailand.

Insect culture

Pulse beetles, *C. maculatus* adults were collected from naturally infested mung bean seeds at the local market, Rat-thakan Road, Hat Yai District, Songkhla Province, Thailand. To establish the stock culture of *C. maculatus,* mung bean seeds (*Vigna radiata* L.) were used as a host. Seeds were kept in an oven for 4 hours at 55ºC for sterilization (Mookherjee et al., 1968). One hundred fifty grams of the sterilized seeds were put in 500 ml plastic containers and 25 pairs of *C. maculatus* adults were released in the containers. The containers were sealed by perforated plastic lids internally lined with muslin cloth to allow for ventilation and preventing the escape of beetles. These parent beetles were allowed to lay egg for 7 days under the laboratory conditions (29 ± 3°C and 75 ± 5% RH) and then they were removed. These containers were stored in the laboratory until adult emergence. One to three day old *C. maculatus* adults were used for all experiments.

Preparation of essential oils

The details of all five plant species used in experiments are given in Table 1. Plant parts were purchased from Trai-buri herbal shop in Hat Yai District, Songkhla Province, Thailand. They were cut into small pieces before extraction by steam distillation through a Clevenger type apparatus.

Table 1. Plant samples evaluated for insecticidal activity against the pulse beetle, *Callosobruchus maculatus*

Scientific name	Common name	Family	Parts used
Syzygium aromaticum	Clove	Myrtaceae	flower bud
Alpinia galanga (L.)	Lengkuas	Zingiberaceae	rhizome
Cinnamomum verum	Cinnamon	Lauraceae	bark
Cymbo pogonnardus	Citronella	Poaceae	leaf
Citrus hystrix DC	Kaffir Lime	Rutaceae	peel

The distillation process was conducted for 5-6 hours and water was eliminated by using anhydrous sodium sulfate. All samples were kept in refrigeration at 4ºC in airtight containers.

Bioassay test by residual contact

Residual contact toxicity of five plant essential oils against adults of *C. maculatus* was investigated by the bioassay method according to Kim et al. (2003), and Usha Rani & Rajasekharareddy (2010). Filter papers were treated with different concentrations of oil solution dissolved in acetone at 8, 16, 24, 32 and 40μl/ml (equivalent to 0.13, 0.25, 0.38, 0.5 and 0.63 $\mu l/cm^2$ filter paper, respectively). A filter paper (9 cm diameter, surface area of 63.6 cm^2) was impregnated in 1 ml of the oil solutions mentioned above by micropipette and placed in a glass Petri dish (9 cm diameter). The control treatment was prepared using only acetone. Acetone was air-dried to evaporate for 10-15 min before releasing five pairs of adult male and female *C. maculatus* into each dish and covered with a lid. The inside of the lid was coated with Vaseline® (pure petroleum jelly) to prevent the insects staying on the lid. All treatments were replicated five times and Petri dishes were kept at room temperature. After treatment for 24, 48 and 72h, insect mortality was recorded.

Seed dressing application

In this application, clean, healthy and uninfected mung bean seeds were used. The clove oil was diluted with acetone and mixed with mung bean seeds at the ratios of 0.4, 0.8, 1.2, 1.6 and 2 ml/kg seeds. A 500 ml conical flask containing 100g of mung bean seeds and oil solution were shaken manually for about two minutes until the seeds were uniformly coated with the oils (Talukder & Howse, 1994). The treated seeds were taken out from the flask and air-dried for one hour to complete evaporation of solvent. Then 100 g seeds were filled into the small jute bag (12 x 10 cm). Thereafter, five pairs of *C. maculatus* adults were released in this bag. These bags were put into the plastic containers (17 x 12 x 7 cm) and sealed with perforated plastic lids internally lined with transparent voile type fabric to allow ventilation and prevent other insects from entering. The control was prepared using acetone only. The experiment was replicated four times and arranged following a completely randomized design (CRD). All containers were kept under laboratory conditions (29 ± 3°C and 75 ± 5% RH). Mortality of adult insects was recorded every 24 hours for 5 days from each bag. Insects that did not respond to being probed three times with a blunt dissecting probe were considered dead (Udo et al., 2011). After seven days, the adults were removed from each bag and stored for one month under the same experimental conditions to record the following data.

Number of F1 adult emergence was observed from each bag after one month.

Percentage reduction in adult emergence or inhibition rate (% IR) was calculated as

$$IR (\%) = [(Cn - Tn) / Cn] \times 100,$$

where Cn = Number of insects in control bag; Tn = Number of insects in treated bag.

Percentage of seed damage and weight loss was observed at the end of the experiment and calculated using the following formula (Boxall, 1986).

$$\text{Seed damage rate } (\%) = [Nd / (Nd + Nu)] \times 100$$

$$\text{Seed weight loss } (\%) = [(U \times Nd) - (D \times Nu)] / U (Nu + Nd) \times 100,$$

where Nd = number of insect damaged seeds; Nu = number of undamaged seeds; U = weight of undamaged seeds; D = weight of insect damaged seeds.

Data analysis

In the present study, corrected mortality percentage was calculated using Abbott's formula (Abbott, 1925). Probit analysis (Finney, 1971) was performed to calculate the lethal concentration for 50% (LC_{50}) insect mortality. Data from seed dressing application, F1 progeny emergence, inhibition rate, seed damage and weight loss of seeds were transformed by using arcsine transformation in order to homogenize the variance (Gomez and Gomez, 1984). All data were subjected to analysis of variance (one-way ANOVA) and significant differences among treatments means were compared at 0.05 significant level using Tukey's Test. All statistical analyses were run on SPSS program (version 23.0).

Results

Bioassay tests by residual contact

The results of residual contact toxicity tests, i.e., insect mortality percentage, and the lethal concentrations of the five plant essential oils, at different concentrations, on *C. maculatus* are shown in Table 2 and Table 3. Clove essential oil caused 96% mortality at concentration as low as 24 µl/ ml, and LC_{50} value 16.09 µl/ ml, after 24 h exposure. This was followed by cinnamon, lengkuas and citronella oils with 84%, 64% and 50% of insect mortality, respectively. The LC_{50} values of these three essential oils were 17.10 µl/ ml, 21.98 µl/ ml and 23.01 µl/ ml after 24h, respectively. The mortality of insects by kaffir lime oil, concentration 24 µl/ml, after 24 h exposure, was not significantly different from the control, while the LC_{50} value was 35.81 µl/ ml. The five tested plant essential oils led to 100% mortality at the high concentration of 40 µl/ ml, and 72h exposure.

Table 2. Residual contact LC_{50} values of the five plant essential oils against the pulse beetle, *Callosobruchus maculatus*

Essential Oils	Residual Contact Toxicity Test LC_{50} (µl/ml)		
	24 h	48 h	72 h
Clove	16.09	12.99	7.67
Cinnamon	17.10	13.12	11.08
Lengkuas	21.98	18.25	12.77
Citronella	23.01	20.97	17.07
Kaffir Lime	35.81	28.01	27.41

Seed dressing application

The percentages of mortality of *C. maculatus* are shown in Table 4. Mortality reached 100% in the treatment level of 2 ml/kg after 5 days of exposure. This was followed by dosage rates of 1.6, 1.2 and 0.8 ml/kg presenting 87.50, 72.50 and 57.70% mortality, respectively, after 5 days of exposure. Mortality at the lowest concentration of 0.4 ml/kg was not significantly different from the control throughout the exposure time. The mortality in the control treatment ranged 0% after 1 day, to 7.50% after 5 days.

The results of clove essential oil treatment on mung bean seed are shown in Table 5. For F1 progeny emergence, the highest number of progenies was recorded in the control, 245.50 ± 16.86, and the lowest number, 2.75 ± 2.14, was in the clove oil treatment, with a concentration of 2 ml/kg. Progeny production significantly decreased with increased dosage of clove oil. Regarding the inhibition rate of clove oil, the highest percentage of inhibition of F1 emergence, 85.94 ± 2.61, was observed with the higher concentration

Table 3. Residual contact toxicity of the five plant essential oils against the pulse beetle, *Callosobruchus maculatus* after 24, 48 and 72 h exposure times

Essential Oils	Concentration, µl/ml	Mean % mortality ± S.E		
		24 h	48 h	72 h
Clove	8	36.00 ± 2.45c	56.00 ± 5.10c	70.00 ± 3.16^{b}
	16	68.00 ± 3.74b	84.00 ± 2.45b	94.00 ± 2.45a
	24	96.00 ± 4.00a	100.00 ± 0.00a	100.00 ± 0.00a
	32	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
	40	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
	Control	0.00 ± 0.00d	0.00 ± 0.00d	0.00 ± 0.00c
Lengkuas	8	18.00 ± 3.74d	30.00 ± 4.47d	58.00 ± 2.00c
	16	38.00 ± 3.74c	58.00 ± 3.74c	84.00 ± 2.45b
	24	64.00 ± 2.45b	82.00 ± 5.83b	100.00 ± 0.00a
	32	88.00 ± 3.74a	100.00 ± 0.00a	100.00 ± 0.00a
	40	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
	Control	0.00 ± 0.00e	0.00 ± 0.00e	0.00 ± 0.00d
Cinnamon	8	28.00 ± 3.74d	48.00 ± 3.74c	66.00 ± 5.10b
	16	62.00 ± 3.74c	80.00 ± 4.47b	90.00 ± 4.47a
	24	84.00 ± 4.00b	100.00 ± 0.00a	100.00 ± 0.00a
	32	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
	40	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
	Control	0.00 ± 0.00e	0.00 ± 0.00d	0.00 ± 0.00c
Citronella	8	0.00 ± 0.00e	10.00 ± 3.16d	22.00 ± 3.74c
	16	18.00 ± 3.74d	24.00 ± 2.45c	54.00 ± 5.10b
	24	50.00 ± 3.16c	70.00 ± 3.16b	92.00 ± 3.74a
	32	78.00 ± 3.74b	100.00 ± 0.00a	100.00 ± 0.00a
	40	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
	Control	0.00 ± 0.00e	0.00 ± 0.00e	0.00 ± 0.00d
Kaffir Lime	8	0.00 ± 0.00c	0.00 ± 0.00d	6.00 ± 2.45de
	16	0.00 ± 0.00c	6.00 ± 2.45d	14.00 ± 2.45d
	24	8.00 ± 3.74c	22.00 ± 3.74c	30.00 ± 3.16c
	32	30.00 ± 3.16b	58.00 ± 3.74b	72.00 ± 4.90b
	40	68.00 ± 4.90a	100.00 ± 0.00a	100.00 ± 0.00a
	Control	0.00 ± 0.00c	0.00 ± 0.00d	0.00 ± 0.00e

Means followed by the same letter(s) in the same column are not significantly different (P > 0.05) from each other using Turkey's Test

Table 4. Mortality percentage of *Callosobruchus maculatus* adults after 1,2,3,4 and 5 days on mung bean seeds treated with different concentrations of clove oil

Dose of clove oil, ml/kg	Insect mortality, % means ± S.E				
	Residual contact				
	Day 1	Day 2	Day 3	Day 4	Day 5
0.4	2.50 ± 2.50c	12.50 ± 2.50c	15.00 ± 2.89de	20.00 ± 4.08d	22.50 ± 4.79d
0.8	12.50 ± 2.50bc	17.50 ± 4.79bc	30.00 ± 4.08cd	45.00 ± 5.00c	57.50 ± 6.29c
1.2	30.00 ± 7.07ab	37.50 ± 7.50ab	47.50 ± 7.50bc	60.00 ± 4.08bc	72.50 ± 2.50bc
1.6	35.00 ± 6.45a	45.00 ± 8.66a	57.50 ± 8.54ab	75.00 ± 6.45ab	87.50 ± 4.79ab
2	42.50 ± 4.79a	52.50 ± 4.79a	77.50 ± 4.79a	92.50 ± 4.79a	100.00 ± 0.00a
Control	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00e	5.00 ± 2.89d	7.50 ± 2.50d

Means followed by the same letter(s) in the same column are not significantly different (P > 0.05)

of 2 ml/kg. This was followed by 77.77 ± 2.48 and 74.80 ± 2.30 percent of inhibition at 1.6 and 1.2 ml/ kg dose levels, respectively. The lower inhibition rates of 60.26 ± 2.76 and 54.14 ± 1.77 were recorded at 0.8 and 0.4 ml/kg of concentrations, respectively. Grain damage and weight loss are the main forms of direct loss inflicted by *C. maculatus* on stored mung bean. The highest seed damage percentage, 23.88 ± 1.17, and the highest weight loss, 5.54 ± 1.09, were observed in the control. The lowest seed damage percentage, 1.64 ± 1.07, and weight loss 0.29 ± 0.17, were observed in the clove oil treatment with the concentration at 2 ml/kg on mung bean seed. Percentage of seed damage, and weight loss of mung bean seeds, significantly decreased with a rise of clove oil concentration.

Discussion

Bioassay test by residual contact

In residual contact toxicity experiments, clove oil had the most effective contact toxicity on adults of *C. maculatus*. This was followed by cinnamon, lengkuas, and citronella oils, which had moderate toxicity effects on adult *C. maculatus*. As shown in Tables 1 and 2, the results of LC_{50} values and insect mortality percentage, kaffir lime oil had the least effect of contact toxicity on tested insects. However, all selected plant essential oils significantly affected the mortality percentages of adults of *C. maculatus* as compared with the untreated control. The results of this study are similar to Mahfuz & Khalequzzaman (2007), who also reported the toxic effects of clove oil, cinnamon oil, cardamom oil, eucalyptus oil and neem oil on *C. maculatus*. The insecticidal toxicity followed in the order: clove> cinnamon> cardamom> neem> eucalyptus after 24h and 48h, respectively. In related research, clove oil and jojoba oil led to the highest mortality percentages of *C. maculatus* adults, followed by rosemary, eucalyptus and citronella oil (Abdullah et al., 2017).

Seed dressing application

Results showed that insect mortality percentage progressively increased with increasing concentration and exposure time. Similarly, Darwish (2016) reported that mortality of *Sitophilus granarius* was directly related to the concentration of five plant oils (clove, anise, matricaria, garlic and celery) and exposure time. Likewise, Arannilewa et al. (2006) reported an increase in adult mortality with increasing time of exposure in all concentrations of four medicinal plant oils (gaping Dutchman's pipe, garlic, sandpaper leaf and bitter kola) against the maize weevil, *Sitophilus zeamais* in the laboratory. The present study agrees with Albandari (2015), who indicated that clove oil was highly effective against the pulse beetle *C. maculatus* at high concentrations (5 mg/L).

Table 5. Adult emergence and seed losses due to seed treatment effects of clove oil against the pulse beetle *Callosobruchus maculatus*

Dose of clove oil, ml/kg	F1 adult emergence	Inhibition rate, %	Seed damage rate, %	Weight loss, %
0.4	85.25 ± 10.70b	54.14 ± 1.77c	15.07 ± 0.90b	2.60 ± 0.27b
0.8	60.50 ± 9.68b	60.26 ± 2.76c	12.09 ± 1.29b	1.93 ± 0.42bc
1.2	17.00 ± 3.87c	74.80 ± 2.30b	6.19 ± 0.73c	1.32 ± 0.11bc
1.6	13.00 ± 4.81c	77.77± 2.48ab	5.20 ± 1.20c	0.94 ± 0.29bc
2	2.75 ± 2.14c	85.94 ± 2.61a	1.64 ± 1.07c	0.29 ± 0.17c
Control	245.50 ± 16.86a	–	23.88 ± 1.17a	5.54 ± 1.09a

Means followed by the same letter(s) in the same column are not significantly different (P > 0.05)

In present study, five different concentrations of clove oil significantly reduced F1 progeny emergence, in comparison with control samples. Furthermore, clove oil caused significant inhibition of F1 progeny among tested insects. In another study, Jumbo et al. (2014) indicated that clove and cinnamon essential oils, at the dose 70.5 µl/kg, significantly suppressed adult emergence of bean weevil, *Acanthoscelides obtectus*. As a result, the weight loss of treated seeds was lower than that of the control. There was a correlative reduction in the number of exit holes in treated mung bean seeds, as a result of insecticidal action of clove oil causing high mortality of adults, thereby less egg laying, and ultimately lower seed damage and weight loss. Other researchers found that bioactivity of neem, castor, karanj, mustard, and groundnut oils significantly reduced seed damage rate by *C. maculatus* on pigeon pea seed (Lolage & Patil, 1992). The results of this study are similar to the findings of Musa et al. (2013) who revealed that F1 adult emergence and seed weight loss depended on the insect population.

In addition, the results obtained from the adult mortality assessment clearly indicate that clove oil is effective against *C. maculatus,* but high concentration and long exposure time are needed to achieve satisfactory to complete control of this pest in mung bean seed. Higher dose rates could restrain the F1 progeny production, inhibition rate, seed damage rate and weight loss in treated mung bean seeds.

Conclusion

The present study indicated that among the five selected plant essential oils, clove oil is the most effective at the lower concentration in residual contact method. Clove oil exhibited a significant toxic effect on *C. maculatus* under storage conditions of mung bean seeds. Clove oil can be used as an alternative to synthetic insecticides for the management of *C. maculatus* in the storage of mung bean seeds. This information will be very useful for farmers in developing countries to control stored product pests, especially *C. maculatus*.

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