

**Chemical Composition and Insecticidal Activities of Essential Oil of
Aegle marmelos (L.) Correa. Against *Tribolium castaneum* Herbst
(Coleoptera: Tenebrionidae)**

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Abstract

Certain compounds of plants such as essential oils, with insecticidal properties have been considered as alternatives to chemical pesticides for pest control. This study intended to determine chemical composition of essential oil extracted from *Aegle marmelos* (L.) Correa. and to evaluate its insecticidal activity against stored-product insect pest, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) adults. Fresh leaves of the plant were extracted using essential oil steam distillation apparatus and analyzed by a gas chromatography-mass spectrometry (GC-MS). Experiment was performed under completely randomized design (CRD) with 4 replications. It was carried out at $30\pm 2^\circ\text{C}$ and $70\pm 5\%$ relative humidity in darkness. Insecticidal activity was investigated on adult of *T. castaneum* by using impregnated filter paper test with 5 concentrations of essential oil including 0%, 4%, 8%, 12%, 16%, and 20% respectively. Data of deaths were recorded after treating at 24 to 168 hours. Total of 120 components were identified and the main compounds in the essential oil of *A. marmelos* were 2,7-dimethyl-3-octen-5-yne (22.63%), limonene (12.29%), santolinatriene (11.42%), p-mentha-1,5,8-triene (7.97%), 2-hexenal, (e)- (7.34%), beta-pinene (4.38%), beta-myrcene (3.26%), beta-phellandrene (2.74%), ethanol, 2-methoxy-, acetate (2.58%), alpha-phellandrene (2.01%), beta-elemene (1.98%) and delta-elemene (1.24%). Insecticidal activity presented 20% of *A. marmelos* essential oil at 120 hours had the highest effective against *T. castaneum* when compared with acetone as control. The results of this study indicate that essential oil of *A. marmelos* leaves have potential to be used in the control of *T. castaneum*.

Keywords: Essential oil, chemical composition, insecticidal activity, stored-product insect control

Introduction

Global post-harvest grain losses from insect damage and other bio-agents range from 10% to 40% [1]. Small-scale farmers may lose as much as 80% of their stock due to insects after storing for 6-8 months [2,3]. Primary factor in these losses is the depredation of stored-product beetles [4,5]. The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), is one of the most widespread and destructive primary insect pests of stored grains and grain products in the tropics [6]. Infestations of grain storage insects not only cause great losses through consumption of grains but also result in raised temperature and moisture conditions that lead to accelerated growth of mold, including toxigenic species [7]. Methods used to control stored grain insect pests included physical, chemical, and biological treatments [8]. Infestations of grain storage insect pests can be controlled by synthetic insecticide fumigants such as organophosphates, pyrethroids, and fumigants (mainly phosphine and methyl bromide) [9,10], which are used both to increase yields and protect stored product. However, repeated use and excessive reliance on fumigant insecticides have exacerbated ozone depletion and environmental pollution. Costs of application and pesticide residue in food have increased while insects have developed insecticidal resistance with toxicity hazards on non-target organisms in addition to direct toxicity to users [8][11,12]. These problems have highlighted the need to develop new types of selective insect-control alternatives with fumigant action. Growing interest in the use of plant extracts for protection of agricultural products has resulted from their low mammalian toxicity and reduced persistence in the environment [1].

Recently, studies have focused on the use of active natural products from various plant essential oils and their bioactive chemical constituents as possible alternatives to synthetic insecticides [13]. One alternative to synthetic pesticides is the use of natural compounds such as essential oils resulting from secondary metabolism in aromatic plants. These oils are typically liquid at room temperature, lipophilic in nature, have densities less than water, and contain a number of bioactive components [14-16]. Plant

essential oils are recognized for their pesticidal properties, both herbicidal [17] and insecticidal [18], as well as their repellent [8][19] and antioxidant [20] activities. They are generally considered safe as an alternative to stored grain conventional pesticides because of their low toxicity to warm-blooded mammals, high volatility [21,22], and rapid biodegradation [23]. Most terpenoids and phenols found in plant essential oils have low toxicity, with some approved as flavoring agents in food [13][24]. Toxicity of many plant essential oils and their constituents has been evaluated against a number of stored-product insects and mites [13][25-27]. Previous studies have assessed the toxic effects of few essential oils to determine possible fumigant, contact and ingestion activity against *T. castaneum* [28,29]. Investigations in several countries have confirmed that some plant essential oils not only repel insect/mites but exhibit contact and fumigant toxicity against stored-product pests as well as possessing feeding inhibition or harmful effects on the reproductive system of insects [8].

Aegle marmelos, commonly known as Bael and belonging to the family Rutaceae is a species of tree native to the Indian subcontinent and Southeast Asia. It is present in Sri Lanka, Thailand and Malaysia as a naturalized species. It is an important medicinal plant in the traditional Indian system of medicine, the Ayurveda. Scientific studies have corroborated many of Bael's ethnomedicinal properties and its potential antimicrobial effects, hypoglycemic, astringent, antidiarrheal, antidysenteric, demulcent, analgesic, anti-inflammatory, antipyretic, wound-healing, insecticidal, and gastroprotective properties [30]. Leaf extract from *A. marmelos* has been found to have insecticidal activity against the brown plant hopper (*Nilaparvata lugens* Stål), an important pest of rice plant in Asia [31] and pulse beetle (*Callosobruchus chinensis* L.) in stored green gram seed (*Vigna radiata* L.) [32]. This study determined the chemical composition of essential oils from *A. marmelos* (L.) Correa. fresh leaves and investigated their insecticidal activities against the adult stage of *T. castaneum*.

Materials and methods

Insect rearing

Adults of the red flour beetle *T. castaneum* were obtained from infested stored seeds collected from Maha Sarakham Province, Thailand. A colony of *T. castaneum* was started with 30 adults. The colony was reared and maintained on wheat flour mixed with wheat and yeast (13:1 w/w) in a plastic bottles (diameter 23 cm, height 30 cm) covered with a fine mesh cloth for ventilation at 30 ± 5 °C, $70\pm 5\%$ relative humidity, and 16:8 hours light/dark cycle for the development of progenies. Adults which emerged after 7 days were used to evaluate the mortality test of essential oil, with all experiments conducted under the same environmental conditions.

Extraction of essential oil

Essential oil was extracted from *Aegle marmelos*, were collected around the Maha Sarakham local in the Northeast of Thailand. Bring fresh leave were washed and air dried in the shade. Using a Clevenger-type apparatus, the extraction of the essential oil was performed from fresh leaves (200 g of an air-dried sample with 600 ml of distilled water), were steam distilled for 3 hours at the Department of Agricultural Technology, Faculty of Technology, Mahasarakham University. Essential oil was dehydrated with anhydrous sodium sulfate to remove water after the extraction and was kept into a vial with the lid closed in the dark at 4 °C until required for use.

Essential oil analysis

Essential oil volatile constituents from *Aegle marmelos* were established by gas chromatography-mass spectrometry (GC-MS) analyses performed on a Clarus SQ 8 GC/MS system (PerkinElmer, MA, USA) operating in EI mode (70 eV). A Rtx-5MS capillary column (with a 5% phenyl-methylpolysiloxane stationary phase, 30 m x 0.25 mm, 0.25 µm film thickness) was used. GC settings were as follows: initial oven temperature was kept at 50 °C for 1 min and then increased to 180 °C at a rate of 10 °C/min, held for 1 min, and then increased at 3 °C/min to 240 °C for 15 min. Injector

temperature was maintained at 230 °C. Samples (1 µL, dilute to 1% with acetone) were injected with a split ratio of 1:10. The carrier gas was helium with a flow rate of 1.0 mL/min. Spectra were scanned from 45 to 450 m/z. Identification of essential oil components was undertaken firstly by comparing their mass spectra with those stored in the National Institute of Standards and Technology (NIST) Mass Spectral Search Program and ChemStation Wiley Spectral Library. Essential oil components were identified by comparison of their retention times with authentic samples to a series of n-alkanes under the same operating conditions.

Mortality test

A series of dilutions of essential oil from *Aegle marmelos* (0, 4, 8, 12, 16 and 20%) was prepared using 100% acetone as solvent as described. An aliquot of each dilution (1 mL) was separately applied on the top surface of a filter paper (diameter 9 cm). The solvent was allowed to evaporate for 5 min before placing into each Petri dish (diameter 9 cm). Ten female adults of *T. castaneum* (7 days) were introduced separately into each Petri dish. The number of dead and live insects in each Petri dish was observed after incubation at 30±5 °C, 70±5% relative humidity, 16:8 hours light/dark cycle and 24 hours to 168 hours exposure. Insects were considered to be dead if no sign of leg or antennal movements were detected. A control experiment was performed whereby treatment involved 100% acetone alone. Each set of treatments was repeated four times. Percentage mortality was determined as described in previous experiment and calculated using the Abbott formula.

Data analysis

Percentage mortality was determined as described in previous experiment and calculated using the Abbott formula [33]. Data were subjected to statistical analysis using CRD design by one-way analysis of variance (ANOVA). Means were compared by using Duncan's Multiple Range Test (DMRT).

Results and discussion

Identification of compounds

Steam distillation of *A. marmelos* fresh leaves afforded essential oil yield of 0.05%. GC–MS analysis led to identification and quantification of 120 constituents. The main compounds were 2,7-dimethyl-3-octen-5-yne (22.63%), limonene (12.29%), santolinatriene (11.42%), p-mentha-1,5,8-triene (7.97%), 2-hexenal,(e)- (7.34%), beta-pinene (4.38%), beta-myrcene (3.26%), beta-phellandrene (2.74%), ethanol,2-methoxy-,acetate (2.58%), alpha-phellandrene (2.01%), beta-elemene (1.98%) and delta-elemene (1.24%), respectively (Table 1). Chemical analysis indicated limonene as the main component of *A. marmelos* essential oil, According to Kim and Lee [34] that showed limonene and beta-pinene were able to kill maize weevil and red flour beetle. Limonene, alpha-pinene and beta-pinene have been known for their toxicities against stored product insects [35-39]. Variation in the composition of essential oils depends on genetics, type and age of leaf source, environment and oil analysis method [28].

Table 1. Chemical composition of essential oils from fresh leaves of *Aegle marmelos*.

No.	Compounds	Formula	Retention Time (min)	Area%
1	ethanol,2-methoxy-,acetate	C ₅ H ₁₀ O ₃	1.477	2.58
2	2-hexenal,(e)-	C ₆ H ₁₀ O	2.929	7.34
3	santolinatriene	C ₁₀ H ₁₆	3.989	11.42
4	beta-phellandrene	C ₁₀ H ₁₆	4.575	2.74
5	beta-pinene	C ₁₀ H ₁₆	4.679	4.38
6	beta-myrcene	C ₁₀ H ₁₆	4.805	3.26
7	alpha-phellandrene	C ₁₀ H ₁₆	5.127	2.01
8	2,7-dimethyl-3-octen-5-yne	C ₁₀ H ₁₆	5.313	22.63
9	p-mentha-1,5,8-triene	C ₁₀ H ₁₄	5.538	7.97
10	limonene	C ₁₀ H ₁₆	5.631	12.29
11	delta-elemene	C ₁₅ H ₂₄	12.975	1.24
12	beta-elemene	C ₁₅ H ₂₄	14.374	1.98

Table 2. Mortality of red flour beetle *T. castaneum* with contact toxicity treated essential oil of *Aegle marmelos* at 24, 48, 72, 96, 120, 144 and 168 h.

Conc. (%)	Mean (\pm SE) mortality (%) of <i>T. castaneum</i>						
	24 h	48 h	72 h	96 h	120 h	144 h	168 h
0	0.00 \pm 0.00	0.00 \pm 0.00 ^c	0.00 \pm 0.00 ^d	0.00 \pm 0.00 ^e	0.00 \pm 0.00 ^e	0.00 \pm 0.00 ^d	0.00 \pm 0.00 ^d
4	0.00 \pm 0.00	0.00 \pm 0.00 ^c	0.00 \pm 0.00 ^d	0.00 \pm 0.00 ^e	0.00 \pm 0.00 ^e	0.00 \pm 0.00 ^d	0.00 \pm 0.00 ^d
8	0.00 \pm 0.00	0.00 \pm 0.00 ^c	0.00 \pm 0.00 ^d	17.50 \pm 5.00 ^d	27.50 \pm 5.00 ^d	32.50 \pm 5.00 ^c	32.50 \pm 5.00 ^c
12	0.00 \pm 0.00	2.50 \pm 5.00 ^c	47.50 \pm 5.00 ^c	47.50 \pm 5.00 ^c	70.00 \pm 0.00 ^c	72.50 \pm 5.00 ^b	80.00 \pm 0.00 ^b
16	0.00 \pm 0.00	27.50 \pm 9.57 ^b	62.50 \pm 5.00 ^b	62.50 \pm 5.00 ^b	90.00 \pm 0.00 ^b	100.00 \pm 0.00 ^a	100.00 \pm 0.00 ^a
20	0.00 \pm 0.00	67.50 \pm 5.00 ^a	80.00 \pm 0.00 ^a	87.50 \pm 5.00 ^a	100.00 \pm 0.00 ^a	100.00 \pm 0.00 ^a	100.00 \pm 0.00 ^a

Means within the same column followed by the same letter are not significantly different (DMRT: P>0.05).

Mortality test

Essential oil of *A. marmelos* was examined against *T. castaneum* adults by the direct contact application method and exhibited 100% adult mortality within 120 h with 20% concentration causing the highest significant difference (P<0.01). Data were reported in terms of percentage mortality with results reported in Table 2. Results indicated that essential oil from *A. marmelos* demonstrated mortality against *T. castaneum* with increased performance at higher concentration levels. The observed results against *T. castaneum* were consistent with previous reports [40,41]. Essential oil present in the leaves of *A. marmelos* was reported for showing insecticidal activity against four stored grain insect pests included *Callosobruchus chinensis* (L.), *Rhyzopertha dominica* (F.), *Sitophilus oryzae* (L.) and *Tribolium castaneum*. In the study grains were infected with test insects, and were fumigated with essential oil of *A. marmelos* (500 μ g/mL). It significantly decreased and controlled crop damage. Meanwhile, it has been reported that essential oil of *A. marmelos* worked as an insect repellent activity against *S. oryzae* and *T. castaneum*. However *A. marmelos* essential oils didn't showed 100% repellent activity against the test insects [42].

Conclusions

Essential oil of *A. marmelos* contains 120 components. The main compound was 2,7-dimethyl-3-octen-5-yne (22.63%). *A. marmelos* displays insecticidal activity against red flour beetle *T. castaneum* and its essential oil has potential of 100% adult mortality. Essential oil from *Aegle marmelos* is an interesting alternative to conventional chemical control strategies and reduces the environmental risks associated with the use of synthetic insecticides. However, further studies are required to evaluate the safety of these oils before practical use in stored-product insect control.

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