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CHEMICAL COMPOSITION AND INSECTICIDAL ACTIVITY OF INDIAN BORAGE ESSENTIAL OIL AGAINST MAIZE WEEVIL

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ABSTRACT: Biopesticide has become more important in stored product pest management, since the use of synthetic insecticides causes adverse effects to human health and the environment. The aim of this research was to determine the chemical composition and toxicity of essential oil from Indian borage, *Plectranthus amboinicu* (Lour.), leaves against adults of maize weevil, *Sitophilus zeamais* Motschulsky. The essential oil was investigated by Gas Chromatograph-Mass Spectrometer (GC-MS) technique. The efficacy of this essential oil against *S. zeamais* using impregnated filter paper and vapor-phase tests. Experiments were performed under Completely Randomized Design (CRD) with 4 replications. Contact toxicity towards maize was investigated at 6 concentrations of essential oil 0 5,000 10,000 15,000 20,000 and 25,000 ppm and fumigant activity was performed using bioassay at 6 concentrations including 0 50 100 150 200 and 250 µL/L air. Data were recorded as number of deaths maize weevil from 24 to 168 h. The principal compounds in the oil were carvacrol (40.49%), caryophyllene (16.76%), Ç-terpinene (11.61%), o-Cymene (8.50%), humulene (5.88%), caryophyllene oxide (2.75), p-mentha-1,4(8)-diene (2.17%), and terpinen-4-ol (1.19%). The essential oil of *P. amboinicu* leaves showed strong contact toxicity at 120 h and fumigant toxicity at 72 h against adults of *S. zeamais* with 100% adult mortality of 25,000 ppm and 250 µL/L air, respectively. The results indicated that the essential oil of *P. amboinicu* leaves showed potent for management of *S. zeamais* population.

Keywords: Chemical composition, Toxicity, Essential oil, Plectranthus amboinicu, Sitophilus zeamais

1. INTRODUCTION

Preservation and protection of stored grain are dietary, economic, and social requirements [1]. Damage caused by insects in stored maize, *Zea mays* L., is a serious problem worldwide. Losses are 10% in developed countries and 20% in developing countries [2]. A major insect pest of stored maize is the maize weevil, *Sitophilus zeamais* Mostchulsky (Coleoptera: Curculionidae) found in moist tropics and subtropics and temperate zones [3]. This specie is generally controlled by insecticides such as phosphine [1]. However, insecticides are toxic to humans and pollute the environment such as ozone depletion, environmental pollution, increasing costs of application, pesticide residue in food, insects develop insecticidal resistance and toxicity hazards on non-target organisms in addition to direct toxicity to users [4], [5]. These problems have highlighted the need to develop new types of selective insect-control alternatives. The use of natural products instead synthetic chemical pesticides is an alternative that can reduce the agriculture impact on the environment.

Insecticidal plants are an alternative to the control of insect pests in stored grains, and can be used to the synthesis of new products or even in the direct control of pests. Secondary metabolites or bioactive compounds present in some botany extracts are toxic to insects by affect its development during the grain colonization [6], [7]. The bioactive compounds can be extracted from leaves, stems, flowers and fruits [7]. In these sense, natural insecticides based on essential oil represent an option for the protection of stored products [8].

Plant essential oils have traditionally been used to kill or repel insects [4] being considered as an alternative to stored grain conventional pesticides because of their low toxicity to warm-blooded mammals, their high volatility [9], [10], rapid degradation [11], and the most terpenoids and phenols found in plant essential oil have low toxicity [12], [1]. Essential oils are effective against insect pests of stored products [13]. Research in recent has been turning more towards selective bio-rational pesticides, that is safer, cheaper and more easy to produce than synthetic insecticides. It has been reported. Essential oil from plants have been proved to possess good potential for use as fumigants against stored product insects including storage bruchids [8], [14]. Some components cause larvicidal effect and oviposition deterrence in agricultural pests [15]. Phenolic compounds in essential oils react with chemical groups of enzymes through hydrogen bonds or hydrophobic interactions, inhibiting activity [16]. Essential oil from wild *L. palmeri* is a good option for controlling maize weevil. The aim of this research were determination of chemical composition of the essential oil of Indian borage, *Plectranthus amboinicu* (Lour.), from fresh leaves and evaluation of the insecticidal activities against adult stage of *S.*

zeamais as a possible alternative for synthetic chemical insecticidal compounds.

2. MATERIALS AND METHODS

2.1 Insect Rearing

Maize weevil, *Sitophilus zeamais* Mostchulsky, from the grain store located in Kantharawichai district, Maha Sarakham Province, Thailand were used throughout this study. Fifteen pairs of adults were maintained in a plastic box (diameter 15 cm, height 30 cm). The cultures were reared on 1 kg of jasmine rice, *Oryza sativa* L. at 30±5°C and 70±5% relative humidity and 16:8 h light/dark cycle and were allowed for mating and oviposition. Adult of maize weevil used for tests were 7 days old.

2.2 Extraction of Essential Oil

Essential oil was extracted from fresh leaves of Indian borage, *Plectranthus amboinicu* (Lour.), using a Clevenger-type apparatus, (200 g of an air-dried sample, 1:3 plant/distilled water ratios) were hydrodistilled for 3 h. Anhydrous sodium sulphate was used to remove water after the extraction. Essential oil was kept in a refrigerator at 4 ºC until its use.

2.3 Experimental Procedure

2.3.1 Analytical essential oil

Essential oil constituents from fresh leaves of Indian borage *P. amboinicu* were established by the gas chromatograph-mass spectrometry (GC-MS) analyses. GC-MS analyses were performed on a PerkinElmer Clarus SQ8 GC/MS system (Roster, USA) operating in EI mode (70 eV). A Rtx-5MS capillary column (with a 5% phenyl-methylpoly siloxane stationary phase, 30 m x 0.25 mm, 0.25 µm film thickness) was used. The GC settings were as follows: the initial oven temperature was kept at 60 ºC for 1 min and increased to 180 ºC at a rate of 10 ºC/min, held for 1 min, and then increased at 3 ºC/min to 246 ºC for 15 min. The injector temperature was maintained at 250 ºC. The samples (1 µL, dilute to 100% 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 50 to 550 m/z. The identification of essential oil components was undertaken firstly by comparing their mass spectra with those stored in National Institute of Standard and Technology (NIST) Mass Spectral Search Program and Chemstation Wiley Spectral Library. Essential oil components were done by comparison of their retention times with authentic samples to a series of n-alkanes under the same operating conditions.

2.3.2 Contact toxicity

A series of dilutions of essential oil from Indian borage *P. amboinicu* (0 5,000 10,000 15,000 20,000 and 25,000 ppm) was prepared using 100% acetone as solvent as described. Aliquot of each dilution (100 µL) was separately applied on top surface of filter paper (whatman no.1, diameter 9 cm) with a micropipette. The solvent was allowed to evaporate for 2 min and placed into each petri-dish (diameter 9 cm). Ten active of female adults *S. zeamais* (7 days) were introduced into the each petri-dish separately. After incubation at 30±5°C and 70±5% relative humidity and 16:8 h light/dark cycle and 24 h exposure to 168 h, adult mortality was recorded. The insects were considered to be dead as no leg or antennal movements were observed. A control experiment was maintained in which treatment was made with 100% acetone alone. Each set of treatment was repeated four times and percentage adult mortality was calculated by using the Abbott formula.

2.3.3 Fumigant toxicity

Whatman (no.1) filter paper strips (1.5x5 cm) were impregnated with 100 µL of 0 (100 % Acetone; control) 50 100 150 200 and 300 µL/L air dilution of essential oils as prepared earlier. After evaporating the solvent for 2 min. Filter paper strips were placed into the hanging of glass vials (diameter 2.5 cm x height 5 cm) from the center of screw cap of fumigation bottle (diameter 5.5 cm x height 10.5 cm) to avoid contact effect of insects with paper strip. Ten active of female adults *S. zeamais* (7 days) were placed inside the fumigation bottle by vapor-phase test. The cap of each bottle was screwed tightly and kept at 30±5 ºC, 70±5% relative humidity, and 16:8 h light/dark cycle. Adult mortality was observed after 24 h exposure to 168 h. The insects were considered to be dead as no leg or antennal movements were detected. A control experiment was maintained in which treatment was made with 100% acetone alone. Each set of treatment was repeated four times and percentage adult mortality was calculated by using the Abbott formula.

3. RESULT AND DISCUSSION

3.1 Identification of Compounds

Chemical composition of essential oil from the leaves of Indian borage, *Plectranthus amboinicu*, is given in Table 1. Total of the components in the essential oil from *P. amboinicu* leaves were 30 compounds as carvacrol (40.49%); caryophyllene (16.76%); Ç-terpinene (11.61%); p-cymene (8.50%); humulene (5.88%);caryophyllene oxide (2.75%); terpinolene (2.17%); α-bergamotene (1.97%); germacra-4(15),5,10(14)-trien-1α-ol (1.28%); terpinen-4-ol (1.19%); á-Myrcene (0.84%); trans-1,2-bis-(1-methylethenyl)cyclobutane (0.40%); 3,7,11, 15-Tetramethyl-2-hexadecen-1ol (0.38%); α-Amorphene (0.37%), 2-Thujene; β-Thujene (0.35%); Humulene epoxide II (0.33%); Linalool (0.28%); Thymol (0.25%); 1-Hepten-3-ol (0.24%); à-Pellandrene (0.23%); Terpinolene (0.13%); Orthodene (0.13%); 11,11-Dimethyl-4,8-dimethyl enebicyclo[7.2.0]undecan-3-ol (0.12%); Ethyltetra methylcyclopentadiene (0.10%); n-Hexadecanoic acid (0.10%); 1,3-Bis-(2-cyclopropyl,2-methylcyclo propyl)-but-2-en-1-one (0.10%); á-Longipinene (0.10%); Cadina-1(6),4-diene (0.10%); 2-isopropyl-5-methylphenol (0.10%) and à-Terpineol (0.10%)

Table 1 Chemical composition of the essential oil from leaves of Indian borage *Plectranthus amboinicu* (Lour.)

No.	Compounds	Retention time (min)	Area%
1	2-Thujene; β-Thujene	8.224	0.35
2	Orthodene	8.534	0.13
3	á-Myrcene	10.068	0.84
4	1-Hepten-3-ol	10.068	0.24
5	à-Pellandrene	11.294	0.23
6	Terpinolene	11.817	2.17
7	p-Cymene	12.172	8.50
8	trans-1,2-bis-(1-methylethenyl)cyclobutane	12.340	0.40
9	Ç-Terpinene	13.688	11.61
10	Terpinolene	14.979	0.13
11	Linalool	15.345	0.28
12	Terpinen-4-ol	19.120	1.19
13	à-Terpineol	19.698	0.10
14	2-isopropyl-5-methylphenol	23.802	0.10
15	Thymol	24.104	0.25
16	Carvacrol	24.775	40.49
17	Caryophyllene	30.059	16.76
18	α-Bergamotene	30.449	1.97
19	Humulene	31.420	5.88
20	α-Amorphene	33.187	0.37
21	Cadina-1(6),4-diene	34.095	0.10
22	Caryophyllene oxide	36.664	2.75
23	Humulene epoxide II	37.655	0.33
24	11,11-Dimethyl-4,8-dimethylenebicyclo[7.2.0]undecan-3-ol	38.624	0.12
25	Germacra-4(15),5,10(14)-trien-1α-ol	39.835	1.28
26	á-Longipinene	41.218	0.10
27	1,3-Bis-(2-cyclopropyl,2-methylcyclopropyl)-but-2-en-1-one	41.463	0.10
28	n-Hexadecanoic acid	49.275	0.10
29	Ethyltetramethylcyclopentadiene	52.818	0.10
30	3,7,11,15-Tetramethyl-2-hexadecen-1ol	54.091	0.38
	Total identified		97.31

Chemical analysis indicated clearly that carvacrol was the main component of Indian borage essential oil. Ortega et al. [17] indicated the chemical constituents varied according to genetic and environmental factors as well as harvest. Erler and Cetin [18] evaluated components and potential larvicidal effects of *Origanum onites* and *O. minutiflorum* against brown-tail moth, *Euproctis chrysorrhoea* (L.), and found carvacrol, thymol, c-terpinene, and terpinen-4-ol, with the major component being carvacrol for both species of oregano. Toxic effects of terpenoid compounds can be attributed to reversible competitive acetyl cholinesterase inhibition occupying the hydrophobic site of the enzyme active site [19].

Carvacrol has insecticidal activity against pests of stored products and is toxic to termites and to nymphs and adults of rice weevil, *S. oryzae*, *Callosobruchus chinensis*, and *Lasioderma serricorne*. Toxic effects in the current study could be attributed to constituents such as thymol, carvacrol, and ρ-cymene. Successful insecticidal plant constituents are monoterpenes and because of volatility, fungicidal, bactericidal, and insecticidal activity might be used to control stored product insects [20].

3.2 Contact Toxicity

Mortality of maize weevil (*S. zeamais*) at 120 h after treatment was highest at the concentration of 25,000 ppm of essential oil from Indian borage (*P. amboinicu*) leaves with cumulative values of 100% and there was a significant difference (P<0.01) in relation to the other concentrations. However, thatwas not different compared with 20,000 ppm (Table 2). The number of dead insects increased as the concentration of the essential oil and time of exposure increased.

3.3 Fumigant Toxicity

Mortality value exposed with 250 μL/L air of essential oil from Indian borage (*P. amboinicu*) leaves to adults of maize weevil (*S. zeamais*) after treatment at 72 h resulted in mortality values 100% and it caused the highest significant difference (P<0.01). However, it was not different compared with 100 150 and 200 μL/L air (Table 3). All concentrations of essential oil had greater efficiency against maize weevil (*S. zeamais*) at 168 h with 100% of adult mortality when comparison with 0 μL/L air, acetone treatments and also was high significant different. Maize weevil (*S. zeamais*) was more sensitive to oil at greater concentrations during the first 72 h and adapted as time passed. Compared with data from this study, Indian borage oil shows greater potential as an insecticide.

Table 2 Mortality of maize weevil *S. zeamais* with contact toxicity treated Indian borage essential oil

Conc. (ppm)	Mean (±SE) of adult mortality (%) of maize weevil *S. zeamais*						
	24 h	48 h	72 h	96 h	120 h	144 h	168 h
0	$0.0±0.0^{d}$	$0.0±0.0^{c}$	$0.0±0.0^{e}$	$0.0±0.0^{e}$	$0.0±0.0^{e}$	$0.0±0.0^{d}$	$0.0±0.0^{c}$
5,000	$2.5±5.0^{d}$	$15±12.6^{b}$	$17.5±5.0^{d}$	$30.0±8.2^{d}$	$40.0±8.2^{d}$	$50.0±8.2^{c}$	$92.5±9.6^{b}$
10,000	$12.5±5.0^{c}$	$20.0±8.2^{b}$	$30.0±8.2^{c}$	$47.5±5.0^{c}$	$60.0±8.2^{c}$	$77.5±5.0^{b}$	$100.0±0.0^{a}$
15,000	$32.5±9.6^{b}$	$55.0±5.8^{a}$	$65.0±5.8^{b}$	$67.5±5.0^{b}$	$72.5±9.6^{b}$	$95.0±5.8^{a}$	$100.0±0.0^{a}$
20,000	$47.5±12.6^{b}$	$80.0±8.2^{a}$	$87.5±5.0^{a}$	$92.5±9.6^{a}$	$92.5±9.6^{a}$	$100.0±0.0^{a}$	$100.0±0.0^{a}$
25,000	$70.0±8.2^{a}$	$77.5±9.6^{a}$	$85.0±5.8^{a}$	$95.0±5.8^{a}$	$100.0±0.0^{a}$	$100.0±0.0^{a}$	$100.0±0.0^{a}$

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

Table 3 Mortality of maize weevil *S. zeamais* with fumigant toxicity treated Indian borage essential oil

Conc. (μL/L air)	Mean (±SE) of adult mortality (%) of maize weevil *S. zeamais*						
	24 h	48 h	72 h	96 h	120 h	144 h	168 h
0	$0.0±0.0^{c}$	$0.0±0.0^{c}$	$0.0±0.0^{c}$	$0.0±0.0^{c}$	$0.0±0.0^{c}$	$0.0±0.0^{b}$	$0.0±0.0^{b}$
50	$10.0±8.2^{b}$	$50.0±14.1^{b}$	$67.5±12.6^{b}$	$77.5±15.0^{b}$	$92.5±9.6^{b}$	$97.5±5.0^{a}$	$100.0±0.0^{a}$
100	$17.5±9.6^{b}$	$70.0±8.2^{ab}$	$87.5±12.6^{a}$	$95.0±5.8^{a}$	$95.0±5.0^{ab}$	$100.0±0.0^{a}$	$100.0±0.0^{a}$
150	$25.0±5.0^{ab}$	$85.0±5.8^{a}$	$87.5±5.0^{a}$	$97.5±5.0^{a}$	$100.0±0.0^{a}$	$100.0±0.0^{a}$	$100.0±0.0^{a}$
200	$17.5±17.1^{b}$	$65.0±28.9^{ab}$	$92.5±9.6^{a}$	$100.0±0.0^{a}$	$100.0±0.0^{a}$	$100.0±0.0^{a}$	$100.0±0.0^{a}$
250	$47.5±15.0^{a}$	$82.5±5.0^{a}$	$100.0±0.0^{a}$	$100.0±0.0^{a}$	$100.0±0.0^{a}$	$100.0±0.0^{a}$	$100.0±0.0^{a}$

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

3.4 Copyright Form

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4. CONCLUSIONS

Indian borage *P. amboinicu* (Lour.) essential oil could be used as a botanical insecticide postharvest since it consists of potential terpenoids such as carvacrol, p-cymene, terpinen-4-ol, and thymol complementary to conventional insecticide. Indian borage essential oil is an alternative control method for maize weevil *S. zeamais* because it results in higher adult mortality by contact and fumigant toxicity. The effectiveness of the components of essential oils might be a viable option to control insect pests in stored maize.

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