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Chemical composition and bioactivity of essential oil from Indian borage (*Plectranthus amboinicus* (Lour.) Spreng) against *Callosobruchus maculatus* (F.)

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Abstract Cowpea weevil, *Callosobruchus maculatus* (F.), is a major insect pest of mungbean causing extensive damage to both quantity and quality. Application of synthetic insecticides to control this insect has a negative effect on human health and the environment. Essential oils from natural plants are recognized as user and environmentally friendly alternatives. Chemical compositions of the essential oil from Indian borage leaves, *Plectranthus amboinicus* (Lour.) Spreng, were analyzed by Gas Chromatography-Mass Spectrometry (GC-MS), and fumigation toxicity of *P. amboinicus* essential oil was evaluated using the vapor-phase test. The main components were carvacrol (71.41%), followed by caryophyllene (7.19%), p-cymene (4.46%), caryophyllene oxide (3.52%), trans-bergamotene (2.53%), humulene (2.26%) and terpinolene (2.16%). The LC_{50} values of fumigation toxicity to *C. maculatus* at 24, 48 and 72 h were 7.18, 5.78 and 5.11 µL/L air, respectively. Killing efficiency of 100% adult mortality of *C. maculatus* was found after 24 h of exposure with 15 µL/L air, while 1 µL/L air of *P. amboinicus* essential oil had repellent effect on adult *C. maculatus* of 87.50% within 48 h. Results showed that *P. amboinicus* essential oil showed a potential as an alternative method to control *C. maculatus* infestations.

Keywords: Lamiaceae, Chemical compounds, Toxicity, Plant essential oils, Stored insect pests

Introduction

Cowpea weevil, *Callosobruchus maculatus* (F.), is a major insect pest of Leguminosae, especially mungbean, and commonly found in bean seed storage areas in the post-harvest period. Adult female weevils lay eggs in mungbean pods from the fields on the surface of the seeds. When the larvae hatch they burrow into the seed and grow until reaching the adult stage. The larvae live in the mungbean seed for about 5 days before mating and the cycle of egg laying begins again. Damaged seeds have white eggs attached to their surface, with at least one round hole per seed caused by an adult emerging from the inside of

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the mungbean seed. The larvae eat the endosperm of the seed until the seed coat remains. This renders the mungbeans unfit for consumption or cultivation (Kanta, 1990). *C. maculatus* has a short life cycle and multiples rapidly.

The popular process of product protection against *C. maculatus* during storage is by applying synthetic insecticides. However, the continuous use of synthetic chemicals in large amounts and for long periods increases insect resistance, making pest eradication more difficult and also increasing the contamination of the environment. Synthetic chemicals also directly and indirectly affect producers and consumers (Emekci, 2010). For these reasons, research interest on the use of essential oils from natural plants to control insect pests has increased. Essential oils are safe and have proven biological activity against insects.

Natural substances from plants, especially essential oils, can both attract and repel insects. When used as repellents they have insecticide, antifeedant and growth regulator properties (Somboon and Pimsamarn, 2003). Essential oils contain secondary metabolites produced by plants (Haque *et al*., 2000) consisting of alkaloids, sesquiterpene, lactone, steroids, phenyl propanes and other compounds that affect insects (Koul and Murray, 1990). Essential oils produced by many families of plants have the ability to control insect pests during product storage. For instance, Zingiberaceae repel maize weevil (*Sitophilus zeamais* Motschulsky) and red flour beetle (*Tribolium castaneum* (Herbst)) (Suthisut *et al.*, 2011), while Asteraceae (Compositae) is effective in protecting against adult and egg stages of *C. maculatus* (Lorestani *et al*., 2013). The Lamiaceae family contains many plant varieties that have shown potential to prevent stored product damage from insect pests (Ayvaz *et al*., 2010).

Indian borage in the family Lamiaceae has the scientific name *Plectranthus amboinicus* (Lour.) Spreng. This plant is commonly seen in the community. Indian borage is a perennial herb that grows to a height of 20-40 cm in 2-3 years. The stem is square to round, succulent and easily broken. The whole plant has a nice aroma. The leaves are light green with an elliptic orbicular shape, acute leaf base and obtuse leaf apex. The thick crispy leaves are covered with short fine hairs and have serrated edges, a convex blade and deep leaf veins. Flowers are inflorescence at the end of the branches. Indian borage is also used as a spice for cooking in Thai and Western food and has medicinal properties (Wichachoo, 2012). The plant also has insecticidal activity. Singh *et al.* (2014) demonstrated that *P. amboinicus* essential oil had effective fumigation toxicity for killing red flour beetle, *Tribolium castaneum*. Other plants belonging to the family Lamiaceae have shown insecticidal properties. Essential oils of rosemary (*Rosmarinus officinalis* L.), marjoram (*Origanum majorana* L.) and oregano (*Origanum vulgare* L.) showed killing effects on *C.*

maculatus (Demirel and Erdogan, 2017). Essential oil of the mint weed (*Hyptis suaveolens*) had contact activity as an insect repellent against *C. maculatus* (Adjou *et al*., 2019), while essential oils of peppermint (*Melissa officinalis*) and sweet basil (*Ocimum basilicum*) were effective for killing, repelling and as an antifeedant on *Sitophillus zeamais* (Yongram *et al.*, 2014). Therefore, the chemical composition, fumigation toxicity, and insecticide and repellent activities of *P. amboinicus* essential oil were evaluated against adult of *C. maculatus*.

Materials and methods

Insect culture

Adults of *C. maculatus* were collected from the mungbean seed storage located in sub-district Khok Phet Phatthana, district Bamnet Narong, Chaiyaphum Province. The insects were cultured to increase their numbers at the Department of Agricultural Technology, Faculty of Technology, Mahasarakham University. Laboratory culture conditions were at 30 ± 5 ℃, 70 ± 5% RH and 8h:16h (light: dark) photoperiod. The cultures were reared on 1 kg of mungbean (*Vigna radiata* (L.) Wilczek). Fifteen pairs of *C. maculatus* adults were selected and placed in a plastic cylindrical container (22 cm diameter, 30 cm height) for 7-10 days to mate and lay eggs. Then, all adults were sieved and removed. Mungbean seeds containing eggs on the surface were separated in a new plastic cylindrical container (11 cm diameter, 11 cm height). After 20 days, the eggs of *C. maculatus* developed into newly emergent adults. Adults of *C. maculatus* used for tests were 5 days old.

Essential oil extraction

Essential oil was extracted from fresh leaves of *P. amboinicus* by the water distillation method. The leaves were first washed and shredded into small pieces for an amount of 200 g. They were then placed in a 2,000 mL round glass bottle and added with 600 mL of distilled water. The bottle was distilled with hot water at 100-120 ℃ for 3 h. All plant parts were totally immersed in the boiling water. The heat caused the water to vaporize and the essential oil in the plant leaves condensed in the condenser and was collected in a glass bulb as the essential oil floating above the water. The essential oil was purified using a centrifuge at a spin speed of 10,000 rpm for 10 min, and *P. amboinicus* essential oil was stored in an amber bottle in a refrigerator at 4 ℃ until required for further analysis.

Identification of chemical compounds

The essential oil constituents from fresh leaves of *P. amboinicu*s were analyzed by a Gas Chromatograph-Mass Spectrometer (GC-MS) series Clarus 680 (PerkinElmer, USA), using a Rtx-5MS capillary column (with a 5% phenyl-methylpoly siloxane stationary phase, 30 m x 0.32 mm, 1.0 µm film thickness). The samples (1 µL, 100,000 ppm) were injected with split mode (split ratio of 1:100 v/v). The carrier gas was helium with a flow rate of 1.0 mL/min. The injector temperature was maintained at 280 ℃. Initial oven temperature was kept at 45 ℃ for 5 min, then increased to 200 ℃ at a rate of 10 ℃/min and held for 5 min, operating in electron impact mode (70 eV). A mass analyzer was used as a quadrupole. The temperature detector was set at 250 ℃. Spectra were scanned (m/z) from 40 to 1,000 amu. Identification of essential oil components was undertaken by comparing their mass spectra with those stored in the National Institute of Standards and Technology (NIST) Mass Spectral Search Program and the Chemstation Wiley Spectral Library. Essential oil components were determined by comparing the substances with mass spectra of substances with quality match more than 80%. Chemical composition data of essential oil from *P. amboinicus* leaves were analyzed by reading the retention time and %area.

Fumigation toxicity

Fumigation toxicity of *P. amboinicus* essential oil on *C. maculatus* was investigated using the vapor-phase test in the sealed fumigation bottles (5.5 cm diameter, 10.5 cm height). There were four replications with six concentrations under 30 ± 5 ℃, 70 ± 5% RH and 8h:16h (light: dark) photoperiod. Essential oil solutions were prepared at concentrations of 0, 3, 6, 9, 12 and 15 µL/L air by dilution with 100% acetone. An aliquot of 100 µL of essential oil solution was dropped on the filter paper (1.5 cm width, 5 cm length) and evaporated at room temperature for 2 min. Each control was prepared with 100 µL of 100% acetone. A filter paper was placed in a small glass bottle (2.5 cm diameter, 5 cm height) and hung at the center of the lid of a fumigation bottle (5.5 cm diameter, 10.5 cm height) with the lid tightly closed. Vapor of the essential oil was produced inside the fumigation bottle. Ten pairs of *C. maculatus* adults (5 days old) were placed in a fumigation bottle and the number of dead *C. maculatus* adults was recorded after exposure for 24, 48 and 72 h. Percentage of adult mortality of *C. maculatus* was calculated using the formula [(NC / NT)] x100, where NC was the number of dead *C. maculatus* adults, and NT was the total number of *C. maculatus* adults used in the test. If the adult mortality rates in the

control were in the range of 5-20%, mortality rates in each method were adjusted by the Abbott's formula (Abbott, 1925). The LC_{50} value of fumigation toxicity of essential oil from *P. amboinicus* leaves on *C. maculatus* adults was determined using Probit analysis.

Insecticidal activity

Insecticidal activity of *P. amboinicus* essential oil against *C. maculatus* was assessed using the vapor-phase test in the sealed fumigation bottles (5.5 cm diameter, 10.5 cm height). Experiments were conducted under Completely Randomized Design (CRD) with four replications at 30 ± 5 ℃, 70 ± 5% RH and 8h:16h (light: dark) photoperiod. Essential oil solutions were prepared at concentrations of 0, 3, 6, 9, 12 and 15 µL/L air and diluted with 100% acetone. An aliquot of 100 µL of essential oil solution was dropped on the filter paper (1.5 cm width, 5 cm length) and allowed to dry by evaporation at room temperature for 2 min. A control was prepared with 100 µL of 100% acetone. A filter paper was placed in a small glass bottle (2.5 cm diameter, 5 cm height), and hung at the center of the lid of a fumigation bottle (5.5 cm diameter, 10.5 cm height) with the lid tightly closed. Vapor of the essential oil was produced inside the fumigation bottle. Ten pairs of *C. maculatus* adults (5 days old) were placed in the fumigation bottle and the number of dead *C. maculatus* adults was recorded after exposure for 24 to 168 h. Percentage of adult mortality of *C. maculatus* was calculated using the formula [(NC / NT)] x100, where NC was the number of dead *C. maculatus* adults, and NT was the total number of *C. maculatus* adults used in the test. If the adult mortality rates in the control were in the range of 5-20%, mortality rates in each method were adjusted by the Abbott's formula (Abbott, 1925). Data were analyzed for the F-test statistic by one-way analysis of variance and means were compared using Duncan's Multiple Range Test (DMRT) at 0.05 probability level ($p < 0.05$).

Repellent activity

The repellent activity of *P. amboinicus* essential oil on *C. maculatus* was assessed by the vapor-phase test. A repellent test kit consists of two plastic bottles (each bottle, 8 cm diameter, 17 cm height) namely as a test bottle and an alternative bottle. There is a hole in the bottom of the side of each bottle for placing a small plastic pipe (0.5 cm diameter, 15 cm length) as a connection between the test bottle and the alternative bottle. A hole was drilled in the middle of the pipe for *C. maculatus* adult release, with a sliding pipe in the center that opened and closed to prevent the escape of *C. maculatus*. The

experiment was conducted under CRD with four replications at 30 ± 5 ℃, 70 ± 5% RH and 8h:16h (light: dark) photoperiod. The essential oil solutions were prepared at six concentrations of 0.5, 1, 1.5, 2, 2.5 and 3 µL/L air by dilution with 100% acetone. An aliquot of 100 µL of essential oil solution was dropped on the filter paper (1.5 cm width, 5 cm length) and evaporated at room temperature for 2 min. A filter paper was placed in a small glass bottle (2.5 cm diameter, 5 cm height) and hung in the center of the lid of the test bottle (8 cm diameter, 17 cm height) with the lid tightly closed. For the alternative bottle, a filter paper was dripped with 100 µL of 100% acetone and prepared in the same way as the test bottle. Ten pairs of *C. maculatus* adults (5 days old) were released into the hole in the middle of the connecting pipe between the test bottle and the alternative bottle and the sliding pipe was tightly closed. The number of *C. maculatus* adults was recorded in the test bottle and the alternative bottle after testing at 24 to 168 h. The percentage of repellent (PR) of *C. maculatus* was calculated using the formula PR = [(NC-NT) / (NC + NT)] x 100, where NC was the number of *C. maculatus* adults found on the side of the alternative bottle, and NT was the number of *C. maculatus* adults found on the side of the test bottle. Data were analyzed for the F-test statistic by one-way analysis of variance and mean values were compared using Duncan's Multiple Range Test (DMRT) at 0.05 probability level ($p < 0.05$).

Results

Components of P. amboinicus essential oil

The 30 chemical constituents of the essential oil from the leaves of Indian borage, *Plectranthus amboinicus* are shown in Table 1. The major components were carvacrol (71.41%), caryophyllene (7.19%), p-cymene (4.46%), caryophyllene oxide (3.52%), trans-Bergamotene (2.53%), humulene (2.26%) and terpinolene (2.16%). Other components of essential oil gave less than 1% area value. Chemical analysis indicated that carvacrol was the main component of *P. amboinicus* essential oil.

Fumigation toxicity

To determine the concentrations of essential oil of *P. amboinicus* that caused death of *C. maculatus* adults, results showed that the fumigation toxicity of *P. amboinicus* essential oil on adults of *C. maculatus* was significantly different, as inferred by the confidence intervals of LC_{50} (Table 2) Essential oil of *P. amboinicus* showed the lowest LC_{50} value and the highest toxicity ratios

for this lethal concentration. A linear regression model gave the best fit for the results of adult mortality, indicating that they were inversely proportional to increasing concentrations of essential oil. Probit analysis showed that *C. maculatus* was more susceptible to *P. amboinicus* essential oil with LC_{50} values at 24, 48 and 72 h of 7.18 (6.47-7.78), 5.78 (4.49-5.85) and 5.11 (3.83-5.16) µL/L air, respectively. Results of linear regression and Probit analysis showed a linear positive relationship between percent mortality and lethal concentration (LC values) of *P. amboinicus* essential oil against *C. maculatus*, as indicated by higher values of regression coefficient (r^2) and slope. Higher values of r^2 indicated that efficacy of the essential oil increased as the level of concentration increased. LC values decreased when *C. maculatus* was exposed to *P. amboinicus* essential oil for a longer period. This indicated that *P. amboinicus* essential oil killed more adults of *C. maculatus* when exposure duration increased from 24 to 72 h.

Table 1. Chemical constituents of essential oil from *P. amboinicus* leaves

No.	Compound	Retention time	%Area
1	3-Hexenol	3.485	0.20
2	3-Octenol	6.252	0.33
3	alpha-Myrcene	6.432	0.19
4	alpha-Terpinene	7.303	0.35
5	p-Cymene	7.731	4.46
6	D-Limonene	7.781	0.07
7	Terpinolene	8.832	2.16
8	Linalool	10.173	0.06
9	2-p-Menthen-1-ol	13.330	0.14
10	trans-sabinene hydrate	13.986	0.19
11	Dihydrocarveol	14.289	0.09
12	Terpinen-4-ol	14.526	0.32
13	(5-Isopropyl-2-methyl-1-cyclopenten-1-yl) methanol	15.239	0.09
14	Carvacrol	20.656	71.41
15	Caryophyllene	23.801	7.19
16	trans-Bergamotene	24.214	2.53
17	Humulene	25.025	2.26
18	alpha-Famesene	25.951	0.07
19	alpha-Muurolene	26.518	0.18
20	alpha-Bisabolene	26.894	0.14
21	Caryophyllene oxide	29.951	3.52
22	Isolongifolol	30.772	0.65
23	Caryophylladienol II	31.685	0.11
24	(1R,7S,E)-7-Isopropyl-4,10-dimethylenecyclodec-5-enol	32.834	0.53
25	Ledene oxide	33.009	0.34
26	Naphthalene, 1,2,3,4,4a,5,6,7-octahydro-4a-methyl-	41.395	0.12
27	Ethyltetramethylcyclopentadiene	45.704	0.06
28	Phytol	47.500	0.14
29	Pregnenolone Acetate	48.609	0.08
30	Neophytadiene	50.538	0.07
	Unknown		1.95
	Total		100.00

Table 2. LC_{50} values of *P. amboinicus* essential oil against adults of *C. maculatus*

Time (h)	n	LC_{50} (95% CL)	y = ax + b	r^2
24	480	7.18 (6.47-7.78)	y = 7.8571x - 6.4286	0.91
48	480	5.78 (4.49-5.85)	y = 7.6548x + 5.7143	0.83
72	480	5.11 (3.83-5.16)	y = 7.0833x + 13.750	0.84

n = number of tested insects (six concentrations, four replications of 20 insects each)
LC = lethal concentration (µL/L air)
CL = confidence limit
r^2 = correlation coefficient

Insecticidal activity

In all cases, significant differences ($p < 0.01$) in adult mortality of *C. maculatus* to the vapor of *P. amboinicus* essential oil were observed at different concentrations and exposure times. Adult mortality increased with rising concentrations from 3 to 15 µL/L air and with exposure time from 24 to 168 h (Table 3). There was no significant difference between concentrations of 12 and 15 µL/L air of *P. amboinicus* essential oil, with respect to its insecticidal activity 24 h after treatment application. In other words, both were different compared with the other concentrations and reached mean adult mortalities for *C. maculatus* of 93.75 ± 2.39% and 100%, respectively. In addition, adult mortalities at 48 to 168 h after treatments at concentrations of 9 µL/L air at 100% were significantly different when compared to 3 and 6 µL/L air. Results showed that adult mortality of *C. maculatus* increased with increasing exposure to *P. amboinicus* essential oil. Thus, there was a response to the increase in concentration. This was perceptible because concentration of 0 µL/L air did not cause adult mortality when evaluated for the same period of exposure.

Table 3. Insecticidal activity of *P. amboinicus* essential oil against *C. maculatus*

Conc.	Adult mortality of *C. maculatus* (%)						
	24 h	48 h	72 h	96 h	120 h	144 h	168 h
0	0.00±0.00^{d}	0.00±0.00^{c}	0.00±0.00^{d}	0.00±0.00^{d}	0.00±0.00^{c}	0.00±0.00^{d}	0.00±0.00^{c}
3	2.50±1.44^{d}	8.75±2.39^{c}	27.50±5.95^{c}	61.25±4.27^{c}	82.50±1.44^{b}	91.25±1.25^{c}	96.25±1.25^{b}
6	33.75±5.15^{c}	70.00±11.37^{b}	73.75±11.97^{b}	76.25±8.98^{b}	88.75±5.15^{b}	96.25±1.25^{b}	96.25±1.25^{b}
9	85.00±5.40^{b}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.0±0.00^{a}	100.00±0.00^{a}
12	93.75±2.39^{ab}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}
15	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}

Conc. = concentration of *P. amboinicus* (µL/L air)
Means within the same column followed by the same letter are not significantly different at $p < 0.5$ by Duncan's Multiple Range Test (DMRT)

Repellent activity

Repellent activity of *P. amboinicus* essential oil against adult *C. maculatus* was 2.5-87.5% with exposure time from 24 to 168 h (Table 4). There was a significant difference ($p < 0.05$) of repellent activity effectiveness of *P. amboinicus* essential oil at concentrations of 0.5-3 µL/L air at 48, 72, 96 and 168 h. Essential oil of *P. amboinicus* at a concentration of 1 µL/L air showed the highest efficacy in repelling adult *C. maculatus* at 48 h with 87.50 ± 5.00%.

Table 4. Repellent activity of *P. amboinicus* essential oil against *C. maculatus*

Conc.	Insect repellence of *C. maculatus* (%)						
	24 h	48 h	72 h	96 h	120 h	144 h	168 h
0.5	60.00±21.21	45.00±9.57^{bc}	52.50±19.31^{a}	17.50±16.52^{b}	25.00±22.17	35.00±18.93	37.50±13.15^{ab}
1	62.50±9.46	87.50±2.50^{a}	80.00±5.77^{a}	60.00±12.25^{a}	52.50±8.54	57.50±9.46	55.00±8.66^{a}
1.5	40.00±10.00	45.00±2.89^{bc}	2.50±2.50^{b}	16.70±15.46^{b}	30.00±18.26	15.00±8.66	10.00±12.25^{b}
2	22.50±11.09	57.50±11.09^{b}	72.50±8.54^{a}	57.50±6.29^{a}	40.00±15.81	42.50±14.93	45.00±12.58^{ab}
2.5	27.50±8.54	12.50±7.50^{d}	17.50±10.31^{b}	7.50±11.09^{b}	15.00±22.17	17.50±14.36	15.00±17.08^{b}
3	42.50±13.15	25.00±9.75^{cd}	12.50±4.79^{b}	2.50±6.29^{b}	20.00±10.80	22.50±6.29	12.50±2.50^{b}

Conc. = concentration of *P. amboinicus* (µL/L air)
Means within the same column followed by the same letter are not significantly different at $p < 0.5$ by Duncan's Multiple Range Test (DMRT)

Discussion

Results of chemical compound analysis concurred with previous reports citing carvacrol as the major constituent in the essential oil of *P. amboinicus* (Senthilkumar and Venkatesalu, 2010; Wanna and Krasaetep, 2019). Major compounds in the essential oil of *P. amboinicus* leaves were 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%) and terpinen-4-ol (1.19%) (Wanna and Krasaetep, 2019). Norazsida *et al*. (2017) found carvacrol at up to 85.14% in the essential oil of *P. amboinicus*, while Hsu and Ho (2019) reported amounts of p-cymene (7.8%), terpinolene (0.3%) and caryophyllene oxide (0.9%). Different quantities and types of chemical components in the leaves may be due to the environment for planting, the harvesting period and diverse extraction methods (Ozcan and Chalchat, 2006). Differences in essential oil compositions were related to the relative proportion of the constituents and not to the presence/absence of a particular component. Variations of chemical constituents may be attributed to genetics, plant parts,

the environment (temperature, photoperiod, and hygrometry) and the harvesting method (Gil *et al.*, 2002; Ortega *et al*., 2011). Carvacrol was found to be effective in killing and inhibiting oviposition of adults of *C. maculatus* (Wahba *et al*., 2018). Likewise, Ajayi *et al.* (2014) reported carvacrol to be effective in killing adults of *C. maculatus*. This may be because carvacrol is a phenolic monoterpene which has a mechanism of action that causes the death of insects. Carvacrol inhibits the enzyme activity of acetylcholinesterase (Oka *et al*., 2000). This affects the structure of invertebrates but the nervous system and the respiratory system are spread throughout the insect body (Ryan and Byme, 1988). Some components of *P. amboinicus* essential oil had % area at less than 1% and can act as insecticides such as alpha-terpinene, alpha-myrcene, D-limonene and linalool (Saglam and Ozder, 2013; Yildirim *et al*., 2013).

Fumigation toxicity showed that longer fumigation periods caused a decrease in LC_{50} value, indicating that *P. amboinicus* essential oil had high fumigation toxicity toward adult *C. maculatus*, as seen from the increased mortality rate. *P. amboinicus* essential oil showed high potential for fumigation toxicity toward adult *C. maculatus* due to LC_{50} values below 8 µL/L air. This result was consistent with the toxicity of hairy basil, *Ocimum americanum*, essential oil in Lamiaceae which showed high LC_{50} fumigation toxicity on adult *C. maculatus* with 0.23 µL/L air (Ilboudo *et al*., 2010). However, by contrast, Esmaili *et al*. (2013) reported that fumigation toxicity of peppermint, *Mentha pulegium*, essential oil in Lamiaceae was 97.3 µL/L air on adult *C. maculatus*. Studies on the mode of action of natural insecticides have shown that treating insects with natural compounds such as essential oils or pure compounds may cause symptoms that indicate neurotoxic activity including hyperactivity, seizures, and tremors followed by paralysis (knock down), which are very similar to those produced by insecticide pyrethroids (Kostyukovsky *et al*., 2002). Essential oils are potent neurotoxins and could affect acetylcholinesterase enzyme inhibition in the central nervous system (Keane and Ryan, 1999). Kellouche *et al.* (2010) reported the efficiency of sage, *Salvia officinalis* L., essential oil in Lamiaceae on adult *C. maculatus*. Essential oil of *S. officinalis* at a concentration of 15 µL/L air gave 100% adult mortality for *C. maculatus*. These authors observed high insect mortality after treatment with *P. amboinicus* essential oil. This effect can be due to the characteristics of the chemical compounds present in the composition of this essential oil, especially carvacrol, one of the main volatile compounds in *P. amboinicus* essential oil (Ajayi *et al.*, 2014; Wahba *et al*., 2018). Toxic effects of *P. amboinicus* essential oil were attributed to its major constituent monoterpenes that are highly volatile and possess high fumigation toxicity. Many plant-derived resources such as monoterpenoids have fumigation activity against a variety of

insect pests attributed to their high volatility (Coats *et al.*, 1991; Shaaya *et al.*, 1997; Ahn *et al.*, 1998). Monoterpenoids (limonene, linalool, terpineol, carvacrol and myrcene) are the main insecticidal constituents of many essential oils, and are effective against stored product insect pests (Regnault-Roger and Hamraoui, 1995). Ahn *et al.* (1998) reported that the monoterpene carvacrol had a wide range of insecticidal activity against various agricultural, stored product and medical insect pests, and also possessed fumigant activity.

Repellent activity of *P. amboinicus* essential oil was reported by Saeidi and Mirfakhraie (2017) who determined the effectiveness of repellent activity of *C. mauclatus* at 87.76 ± 0.96% at 24 h for an experiment conducted with 360 µL/L air of peppermint, *Mentha piperita*, essential oil that is also classified as Lamiaceae. Similarly, we found that concentrations of 1.5 µL/L air at 72 h and 3 µL/L air at 96 h of *P. amboinicus* essential oil gave the lowest repellence percentage of *C. maculatus* at 2.50 ± 2.50% and 2.50 ± 6.29%, respectively.

In the fumigation method, *C. maculatus* showed high susceptibility to *P. amboinicus* essential oil, even at low concentrations and reduced exposure period. Longer exposure was required to obtain 90% toxicity to *C. maculatus*. Toxicity against insects contaminating stored products is influenced by the chemical composition of the essential oil from the used plant part (Lee *et al.*, 2001). Previous reports suggested that monoterpenoids such as carvacrol, alpha-terpinene, linalool, alpha-myrcene, D-limonene, caryophyllene, p-cymene, terpinen-4-ol and humulene, found in the essential oil of *P. amboinicus*, were responsible for insecticidal activity and repellent properties.

Our results indicated the strong insecticidal activity of *P. amboinicus* essential oil and its potential role as a fumigant for adult *C. maculatus*. *P. amboinicus* essential oil has potential for applications in integrated pest management (IPM) programs for stored grain pest control due to its high volatility and fumigant activity. In light of the information obtained, essential oil from the leaves of *P. amboinicus* can be considered an environmentally friendly alternative for stored grain protection and reduce the risks associated with the use of synthetic insecticides. Further investigations are required to increase knowledge for the effective and widespread use of these technologies, especially on a commercial basis. Formulation on large scale production required.

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