Toxicity of Essential Oil from *Plectranthus amboinicus* (Lamiales: Lamiaceae) against *Sitophilus zeamais* (Coleoptera: Curculionidae)[1]

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Abstract Maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), is a major insect pest of stored grain products, causing extensive damage to both grain quantity and quality. The application of synthetic insecticides to control this insect has a negative impact on human health and the environment. Essential oils derived from natural plants are recognized as user- and environmentally friendly alternatives. The use of essential oils from Lamiaceae, which exhibit toxic properties through contact, fumigation, and repellency, provides another option for protecting stored products from various insect pests. The aim of this research was to study the chemical constituents and insecticidal activity of essential oil extracted from the leaves of Indian borage, *Plectranthus amboinicus* (Lour.) (Lamiales: Lamiaceae), against *S. zeamais* adults.The chemical composition of the essential oil was analyzed using gas chromatography–mass spectrometry, and the insecticidal activity against adult maize weevils was investigated in the laboratory. The experimental plan followed a completely randomized design with five replications of seven treatments or concentrations of the essential oil: 0, 0.5, 1, 1.5, 2, 2.5, and 3 μl/ml air. The results revealed that the essential oil from the leaves of *P. amboinicus* contained 23 chemical constituents (97.51%). Thymol (49.96%) was identified as the main compound followed by caryophyllene, trans-α-bergamotene, 3-methyl-4-isopropylphenol, γ-terpinene, p-cymene, caryophyllene oxide, humulene, 4-hydroxy-2-methylacetophenone, 2-hydroxy-2-phenylbutyramide, (2-oxazolidinylidene)malononitrile, hexestrol, terpinen-4-ol, 1-octen-3-ol, (1R,7S,E)-7-isopropyl-4,10-dimethylenecyclodec-5-enol, α-bisabolene, isoaromadendrene epoxide, α-farnesene, 4-carene, α-muurolene, 3-hexen-1-ol, ledene oxide-(II), and α-myrcene, respectively. The fumigation toxicity (median lethal concentration) to maize weevil adults at 48 h was 292.53 μl/ml air. The essential oil from the leaves of *P. amboinicus*, when applied at a concentration of 2 μl/ml air for 144 and 168 h, resulted in adult maize weevil mortality exceeding 90%. The highest concentration tested (3 μl/ml air) led to exceptionally high mortality rates, with 99–100% of the maize weevils succumbing to the treatment. These findings demonstrate that essential oil from *P. amboinicus* leaves holds promise as a fumigation insecticide for reducing the population of maize weevils, a significant pest in stored products.

Key Words chemical composition, toxicity, insecticide, stored-product insect pest, weed essential oil

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The Lamiaceae (formerly Labiatae) consists of shrubs or vines with square stems that emit essential oils. The flowers are inflorescences found along the axils or around the joints, with symmetrical sides. This family includes plants such as basil (*Ocimum basilicum* L.), thyme (*Thymus* spp.), mint (*Mentha* spp.), fennel (*Foeniculum vulgare* Mill.), lavender (*Lavandula spica* L.), rosemary (*Rosmarinus* officinalis L.), blue salvia (*Salvia azurea* Michx. Ex Lam.), red hummingbird (*Agastache cana* (Hook.) Woot. & Standl.), and oregano (*Origanum vulgare* L.). These plants are commonly used as spices in cooking and for flavoring and adding color to food. In addition, they possess medicinal properties and are used as components in medicine. The essential oils of Lamiaceae are also used against many insect pests. For example, thyme (*Thymus daenensis* Celak) essential oil can be used to eliminate cowpea weevil (*Callosobruchus maculatus* (F.)) and the wheat weevil (*Sitophilus granaries* (L.)) (Jarrahi et al. 2016). Similarly, Singh (2016) reported that the rosemary (*Rosmarinus officinalis* L.) essential oil exhibited contact toxicity against cowpea weevil and red flour beetle (*Tribolium castaneum* (Herbst)). Essential oils from rosemary, marjoram (*Origanum majorana* L.), and oregano were found to be effective against cowpea weevil (Demirel and Erdogan 2017), and *Hyptis suaveolens* (L.) Poit essential oil acted as a repellent against cowpea weevil (Adjou et al. 2019). Moreover, Souza et al. (2016) reported that basil essential oil and spearmint (*Mentha spicata* L.) were toxic to lesser grain borer (*Rhyzopertha dominica* (F.)). This efficacy against insect pests can be attributed of the fact that essential oils are secondary metabolites produced by plants (Haque et al. 2000) that contain alkaloids, sesquiterpenes, lactones, steroids, phenyl propanes, and other compounds that act on insects when ingested or consumed (Koul and Isman 1990). Thus, essential oils from the family Lamiaceae offer promising alternatives for controlling or preventing insect pests.

Thailand is rich in natural resources, and many herbal plants have been used in traditional medicine to treat common ailments (Chotchoungchatchai et al. 2012, Maneenoon et al. 2015). The essential oils of some herbal plants contain secondary metabolites that protect against natural pests. The most prevalent among these secondary metabolites are alkaloids, saponins, phenols, and terpenes, all of which possess insecticidal properties (Gajger and Dar 2021). Many plant families produce essential oils that can control insect pests. For example, Asteraceae is effective against various stored insect pests. Essential oils of *Ageratum conyzoides* L., *Chromolaena odorata* (L.) King & Robinson, *Lantana camara* L., *Wedelia trilobata* (L.) A.S. Hitchcock, *Porophyllum linaria* (Cav.) DC., *Tagetes patula* L., *Artemisia sieberi* Besser, and *Achillea millefolium* L. have been effective against *S. zeamais*, *Sitophilus oryzae* (L.), *S. granaries*, and *R. dominica* through the inhibition of oviposition and progeny emergence (Hernández-Cruz et al. 2019, Alkan 2020, Shahinfar et al. 2021, Wanna et al. 2021). By contrast, Apiaceae demonstrates fumigant and repellent potentials of *Daucus carota* L. against adult *S. zeamais* (Wanna et al. 2022) and Rutaceae shows insecticidal activity against egg, larval, and adult stages of the stored grain insect pest *T. castaneum* (Wanna and Satongrod 2020). Lamiaceae is thus another important family producing essential oils with insecticidal activities (Ebadollahi et al. 2020).

Plectranthus amboinicus (Lour.) Spreng, commonly known as Indian borage (Thai name, Niam Hu Suea), is a perennial herb belonging to the Lamiaceae. It is widely distributed throughout the tropics and warm regions of the Old World, in particular Africa, Asia, and Australia (Arumugam et al. 2016). This herb exerts numerous pharmacological properties, including antimicrobial, anti-inflammatory, antitumor, wound-healing, antiepileptic, insecticidal, antioxidant, and analgesic (Arumugam et al. 2016, Wadikar and Patki 2016, Jimmy 2021). This research aimed to study the chemical constituents and insecticidal activity of *P. amboinicus* essential oil against adult *S. zeamais*.

Materials and Methods

Insect rearing. Adult maize weevils were cultured in 10-L plastic buckets containing 500 g of corn (*Zea mays* L.) seeds. Each plastic bucket was populated with 15 pairs of maize weevils. The buckets were stored at room temperature (27 $\pm$ 2°C), and the lids were opened daily to allow air circulation. After 4 d, the adult maize weevils were removed from the plastic buckets, leaving only the maize weevil spawns. These spawns were preserved until the adult maize weevils emerged. Subsequently, the emerging adults were reared until a consistent and sufficient number of maize weevils was obtained for conducting the insecticidal activity bioassay using *P. amboinicus* essential oil. For the test, 7-d-old adult maize weevils were used.

Essential oil extraction. Essential oil of *P. amboinicus* was extracted using a water distillation method. Three kilograms of *P. amboinicus* fresh leaves were cleaned and shredded into small pieces. Next, 200 g of these plant parts were placed into 2,000-ml round flasks and mixed with 1,000 ml of distilled water. This mixture was subjected to a distillation process at 100–120°C for 3 h by using essential oil distillation equipment. After distillation, the essential oil that separated floated atop the water. This layer was purified through centrifugation at 10,000 rpm for 10 min to remove any residual water. The obtained *P. amboinicus* essential oil was stored in amber glass bottles at 4°C until it was further analyzed and tested for insecticidal activity.

Chemical composition analysis. The extracted essential oil was analyzed following the method of Satongrod et al. (2021) with gas chromatography (model Clarus 680 gas chromatograph, PerkinElmer)-mass spectrometry. The column used was an Rtx-5MS capillary type, with a length of 30 m, a diameter of 0.32 mm, and a thickness of 1 μm. For injection, the essential oil was used in split mode (split ratio, 1:100 v/v) at a concentration of 500,000 ppm, with a volume of 1 μl. Helium gas was used as the carrier gas at a flow rate of 1.0 mm/min. The injector temperature was set at 280°C. At column state, an initial temperature of 45°C was maintained for 5 min and then the temperature was increased at a rate of 10°C/min until it reached 200°C, where it was held for 5 min. Electron impact mode mass spectrometry conditions were 70 eV, by using a quadrupole mass analyzer. The detector temperature was set at 250°C. The substances were characterized by spectral comparison using the National Institute of Standards and Technology Mass Spectral Search Program and the Chemstation Wiley Spectral Library. They were considered comparable to known substances with mass spectra that had a quality match of $>$80%. The chemical composition data were analyzed based on the reading of the retention time and percent peak area.

Insecticidal activity bioassay. The fumigation toxicity of *P. amboinicus* essential oil against maize weevil was tested using a vapor phase test in 40-ml glass vials with lids. The experimental plan followed a completely randomized design (CRD) with five replications of seven treatments at concentrations of 0, 0.5, 1, 1.5, 2, 2.5, and 3 μl/ml air. Each concentration was prepared by diluting essential oils in acetone. In each glass vial, we placed 10 g of dried corn kernels. Next, 100 μl of the essential oil

Table 1. Chemical compounds of essential oil from *Plectranthus amboinicus* leaves.

No.	Compound	Retention Time (min)	% Area
1	3-Hexen-1-ol	3.493	0.25
2	1-Octen-3-ol	6.340	0.91
3	α-Myrcene	6.452	0.14
4	4-Carene	7.311	0.37
5	p-Cymene	7.726	3.86
6	γ-Terpinene	8.922	4.26
7	Terpinen-4-ol	14.772	0.94
8	Thymol	20.296	49.96
9	Hexestrol	20.409	2.24
10	4-Hydroxy-2-methylacetophenone	20.454	2.80
11	3-Methyl-4-isopropylphenol	20.542	4.32
12	(2-Oxazolidinylidene)malononitrile	20.589	2.38
13	2-Hydroxy-2-phenylbutyramide	20.629	2.74
14	Caryophyllene	23.942	8.77
15	*trans*-α-Bergamotene	24.415	5.11
16	Humulene	25.165	2.88
17	α-Muurolene	26.579	0.30
18	α-Bisabolene	26.974	0.43
19	α-Farnesene	27.512	0.39
20	Caryophyllene oxide	29.837	3.04
21	Ledene oxide-(II)	32.529	0.21
22	(1R,7S,E)-7-Isopropyl-4,10-dimethylenecyclodec-5-enol	32.862	0.82
23	Isoaromadendrene epoxide	33.037	0.42
	Total		97.51

solution was aspirated using a micropipette and dripped onto a filter paper (Whatman no. 1; diameter, 2 cm). The filter paper was left to evaporate for 5 min before being placed inside the glass vial lid. Next, 7-d-old maize weevil adults were released into each glass vial, with 10 pairs/vial, and the glass bottle caps were tightly closed. The number of dead maize weevils was recorded at 24, 48, 72, 96, 120, 144, and 168 h.

Table 2. Fumigation toxicity of *Plectranthus amboinicus* essential oil against adult maize weevils (*Sitophilus zeamais*).*

Time (h)	*n*	LC_{50} (µl/ml air)	Lower 95% CL	Upper 95% CL
24	600	1,442.39	460.68	4,709.45
48	600	292.53	171.75	282.10
72	600	161.35	2.68	153.90

* *n* is number of weevils tested; CL, confidence limit; LC_{50}, median lethal concentration.

Statistical analyses. Percent mortality of adult maize weevils was evaluated by the formula (Nd/Nt) × 100, where Nd is the number of dead maize weevil adults and Nt is the total number of maize weevil adults used in the bioassay. If the maize weevil mortality in the control treatments fell within the range of 5–20%, mortality for each treatment was adjusted using Abbott's formula (Abbott 1925). The median lethal concentrations (LC_{50}) were determined using probit analysis (Finney 1971). The data were analyzed using one-way analysis of variance according to the CRD design. Treatment means were compared with the least-significant difference (LSD) test ($P < 0.05$) with Statistix 9.0 (Analytical Software, Tallahassee, FL).

Results and Discussion

Chemical composition of *P. amboinicus* essential oil. Analysis of the chemical composition of the essential oil from *P. amboinicus* leaves revealed 23 chemical constituents (97.51%), with thymol (49.96%) identified as the main compound followed by caryophyllene (8.77%), *trans*-α-bergamotene (5.11%), 3-methyl-4-isopropylphenol (4.32%), γ-terpinene (4.26%), p-cymene (3.86%), caryophyllene oxide (3.04%), humulene (2.88%), 4-hydroxy-2-methylacetophenone (2.80%), 2-hydroxy-2-phenylbutyramide (2.74%), (2-oxazolidinylidene)malononitrile (2.38%), hexestrol (2.24%), terpinen-4-ol (0.94%), 1-octen-3-ol (0.91%), (1R,7S,E)-7-isopropyl-4,10-dimethylenecyclodec-5-enol (0.82%), α-bisabolene (0.43%), isoaromadendrene epoxide (0.42%), α-farnesene (0.39%), 4-carene (0.37%), α-muurolene (0.30%), 3-hexen-1-ol (0.25%), ledene oxide-(II) (0.21%), and α-myrcene (0.14%) (Table 1). These finding were consistent with previous reports on the major constituents present in *P. amboinicus* essential oil, such as thymol, carvacrol, 1,8-cineole, p-cymene, and terpinen-4-ol (Murthy et al. 2009, Senthilkumar and Venkatesalu 2010). Singh et al. (2002) reported thymol (93%) as the major constituent, whereas Wanna and Krasaetep (2019) reported carvacrol (40.49%) as the major constituent. The essential oil of *P. amboinicus* contained two major phenolic monoterpenes, namely, carvacrol and thymol, that showed insecticidal activity (Singh et al. 2002, 2014; Wanna and Krasaetep 2019; Wanna and Kwang-Ngoen 2019; Satongrod and Wanna 2020).

Insecticidal activity. The fumigation toxicity (LC_{50}) of essential oil from *P. amboinicus* leaves on maize weevils at 24, 48, and 72 h was 1,442.39, 292.53, and 161.35 µl/ml air, respectively (Table 2). The mortality of adult maize weevils after fumigation with *P. amboinicus* essential oil at seven concentrations showed significant differences ($P < 0.01$) within 48, 72, 96, 120, 144, and 168 h (Table 3),

Table 3. Mortality (mean ± SD) of maize weevils (*Sitophilus zeamais*) exposed to essential oil of *Plectranthus amboinicus* leaves.

Concentrations (μl/ml air)	Mortality of maize weevil adults (%)*					
	48 h	72 h	96 h	120 h	144 h	168 h
0	0.00 ± 0.00d	0.00 ± 0.00c	0.00 ± 0.00d	0.00 ± 0.00e	0.00 ± 0.00e	0.00 ± 0.00d
0.5	14.00 ± 5.83cd	33.00 ± 13.63b	48.00 ± 18.33c	49.00 ± 19.84d	52.00 ± 16.91d	56.00 ± 18.00c
1	19.00 ± 3.74cd	39.00 ± 12.00b	51.00 ± 13.56c	58.00 ± 10.77cd	68.00 ± 6.78cd	78.00 ± 10.77b
1.5	23.00 ± 6.00bc	49.00 ± 19.33ab	61.00 ± 18.54bc	74.00 ± 12.00bc	79.00 ± 12.40bc	86.00 ± 9.69ab
2	42.00 ± 9.27ab	69.00 ± 9.69a	83.00 ± 6.78ab	89.00 ± 3.74ab	91.00 ± 5.83ab	96.00 ± 3.74ab
2.5	42.00 ± 12.48ab	72.00 ± 15.36a	91.00 ± 6.63a	94.00 ± 5.83ab	99.00 ± 2.00a	99.00 ± 2.00a
3	49.00 ± 17.72a	70.00 ± 14.14a	92.00 ± 6.78a	97.00 ± 4.00a	100.00 ± 0.00a	100.00 ± 0.00a
F test	**	**	**	**	**	**
% CV**	21.44	29.78	26.81	22.71	19.40	19.92

* Means within the same column followed by the same letter are not significantly different (LSD test, $P > 0.05$). For the F test, the double asterisk (**) represents a significant difference at $P < 0.01$.

** % CV, percent coefficient of variation.

but not at 24 h. All concentrations of *P. amboinicus* essential oil resulted in <50% mortality of adult maize weevils within 48 h. An essential oil concentration of 2.5 μl/ml air at 96 and 120 h induced >90% mortality of adult maize weevils, and no significant difference was found compared with the highest concentration of 3 μl/ml air. The mortality of adult maize weevils was >90% at 2 μl/ml air within 144 and 168 h, and there was no significant difference compared with concentrations of 2.5 and 3 μl/ml air, causing the mortality of the adult maize weevils to reach as high as 99–100%. The mortality of adult maize weevils increased with a higher concentration of the essential oil solution and a longer duration of exposure. The insecticidal activity of *P. amboinicus* essential oil at a concentration of 3 μl/ml air resulted in a maximum 100% mortality of adult maize weevils within 144 h, which was 30 times higher than the concentration reported by Wanna and Krasaetep (2019), where 0.1 μl/ml air of *P. amboinicus* essential oil achieved 100% efficiency for the same duration of exposure. Satongrod and Wanna (2021) reported that essential oil from *P. amboinicus* at a concentration of 1 μl/L air inhibited cowpea weevil spawning by 70.53% and first-generation adult spawning by 73.30%, which is consistent with the finding of Keita et al. (2000), who stated that *Ocimum* spp. (Lamiaceae) could be used as an alternative to synthetic insecticides against adults and inhibit the egg laying of cowpea weevil. In addition, previous reports indicate that carvacrol, thymol, caryophyllene, humulene, terpinene, cymene, and terpineol in essential oils of *P. amboinicus* exhibit insecticidal, antifungal, and antibacterial properties (Negahban et al. 2007, Bhatt and Negi 2012, Gonçalves et al. 2012, Satongrod and Wanna 2020). These properties may be attributed to the presence of different types and amounts of compounds in essential oils. It is well known that the variability of the chemical compositions of essential oils depends on geography, growing season, genetics, plant age, harvest time, and method of essential oil extraction (Özcan and Chalchat 2006, Ortega et al. 2011). These findings demonstrate the potential for using these natural compounds as an effective alternative to synthetic pesticides.

In summary, the essential oil obtained from *P. amboinicus* leaves in this study was comprised of 23 chemical constituents, with thymol being the most prominent. A fumigation toxicity bioassay demonstrated high effectiveness against adult maize weevils when administered at a concentration of 2 μl/ml air, resulting in substantial mortality within 48–168 h. This underscores the potential of *P. amboinicus*–derived essential oil as a viable insecticidal agent for managing maize weevil populations that pose significant threats to agricultural storage. Given the variability in essential oil compositions, further investigations are imperative to pinpoint the specific compound(s) responsible for its insecticidal properties, thereby ensuring consistent treatment outcomes. Furthermore, a comprehensive elucidation of an essential oil's mechanisms of action on insects and mammals, including the roles played by minor components, is essential before contemplating its commercial development and use.

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