

# Fumigant Activity of *Tridax procumbens* (Asterales: Asteraceae) Essential Oil Against *Sitophilus zeamais* (Coleoptera: Curculionidae) and Its Effects on Thai Rice Seed Germination<sup>1</sup>

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**Abstract** Fumigant activity of the essential oil extracted from *Tridax procumbens* L. (Asterales: Asteraceae) by water distillation was assessed against *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), together with its effect on the germination of three Thai varieties of rice, *Oryza sativa* L. (Poales: Poaceae). *Tridax procumbens* essential oil contains 25 compounds with five principal components as 1,2-cyclooctanediol (11.49%), hexanal (5.34%), 4-heptenal (4.92%), 2,4-nonadienal (3.10%), and 1,6-dimethylhepta-1,3,5-triene (1.67%). *Tridax procumbens* essential oil was a fumigant toxin to *S. zeamais* adults with a median lethal concentration (LC<sub>50</sub>) of 1,509.79 µl/L air 24 h after exposure. The highest concentration of 250 µl/L air of *T. procumbens* essential oil showed low efficacy in killing adults of *S. zeamais* at 24 h with 10% mortality. This concentration also affected newly emerged progeny (F1) of *S. zeamais* compared to the control, with the highest reduction of 92.87%, and also decreased seed germination of the three varieties of Thai rice RD6, Kham Na Sinuan, and KDML at 94.25, 96.0, and 93.25%, respectively, compared with the control (98.0–98.75%). Results indicate that essential oil from *T. procumbens* has potential for application on stored products to control progeny of *S. zeamais* that cause seed damage.

**Key Words** toxicity, chemical compounds, essential oil, stored insect pests, germination

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The maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), is a well-known stored grain insect pest with wide distribution in high temperature and humid conditions (López-Castillo et al. 2018; Nwosu 2018). The larvae and adults of the maize weevil cause significant damage to stored grain. Approximately 50% of the eggs can be oviposited during the first 5 weeks of the adult's life (Tefera et al. 2011). The female chews a hole in the grain and lays eggs inside before sealing the opening with a secretion, thus protecting the eggs within the grain to complete their life cycle. Therefore, most insect control strategies focus on adult control (Patiño-Bayona et al. 2021). Grain infestation by this insect causes weight loss, reduced germination capacity, reduced nutrient content and nutritive qualities, as well as grain off-taste and odor (Barney et al. 1991; Caneppele et al. 2003; Hell et al. 2000). High temperature and humid conditions promote the reproduction of

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other insect pests and microorganisms such as *Aspergillus flavus* Link and various species of bacteria (Fleurat-Lessard 2016; Hubert et al. 2018; Nesci et al. 2011). Paz et al. (2018) determined that insect pests destroy 20–30% of stored products in tropical and subtropical global regions.

Various strategies have been used to control the maize weevil, with chemical control the most effective. A key factor in chemical control is the application mode that depends on the insect ecology as well as the characteristics of the place or product to be treated. The mode of bulk grain storage in warehouses or silos makes it difficult to prevent and control insect infestations (Fleurat-Lessard 2016). Insecticides with fumigant action are the most effective method for controlling pests in stored products because their high volatility can spread throughout the air space of warehouses and silos, impacting areas not readily accessible to sprays (Thoms and Busacca 2016). However, commercial pesticides are often nonselective and many are harmful to worker health and toxic to the environment. Methyl bromide has now been discontinued and banned due to environmental concerns and health hazards (Boyer et al. 2012; Herrera et al. 2015). Phosphine is a fumigation used in stored pest control; however, heavy dependence on phosphine has resulted in resistance development of numerous stored insect pests including *Sitophilus* spp. in grain stores (Nayak et al. 2020) as well as in the food industry and flour mills (Aulicky et al. 2015). Therefore, more-effective and safe alternatives are needed to control stored grain pests.

Botanical plants have recently received increasing attention. They present minor threats to human health and the environment due to their low mammalian toxicity and low environmental persistence (Isman 2006). Plant essential oils are a possible alternative source for pest management. They display varied biological activities, are biodegradable, and have minimal effects on nontarget organisms and the environment (Boyer et al. 2012; Patiño-Bayona et al. 2021; Peschiutta et al. 2019). Essential oils can be extracted from various plant parts such as bark, flowers, buds, leaves, peel, and resin, mostly by steam distillation. Essential oils are complex, biodegradable, volatile, and lipophilic combinations of terpenoids (monoterpenoids and sesquiterpenoids) and phenylpropanoids (Bhavva et al. 2018). Many studies reported that compounds of essential oils cause a toxic effect in insects through contact, ingestion, or fumigation. Biological activities also produce acute and chronic toxicity on other behavioral effects of insects such as repellency, antifeedant, inhibition of oviposition, and growth development (Benelli et al. 2017; Bett et al. 2016; Mossa 2016; Papachristos and Stamopoulos 2002; Reddy et al. 2016). Previous studies have shown that essential oils from various plant families can be used to control maize weevils.

Extracts from members of the Asteraceae plant family are reportedly 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 *Sitophilus* spp. such as the maize weevil (*S. zeamais*), rice weevil (*Sitophilus oryzae* L.), and wheat weevil (*Sitophilus granaries* [L.]) through inhibition of oviposition and progeny emergence (Alkan 2020; Bouda et al. 2001; Hernández-Cruz et al. 2019; Mossi et al. 2011; Shahinfar et al. 2021; Wanna et al. 2021). Essential oils of *Achillea wilhelmsii* C. Koch, *Pulicaria gnaphalodes* Ventenat, *Artemisia judaica* L., and *W.*

*trilobata* were effective in inhibiting oviposition and killing newly emerged progeny of cowpea weevil, *Callosobruchus maculatus* (F.) (Abd-Elhady 2012; Khani and Asghari 2012; Satongrod et al. 2021), while essential oils of *Pulicaria gnaphalodes* Ventenat, *Artemisia judaica* L., *Artemisia absinthium* L., *Artemisia campestris* L., and *Artemisia herba-alba* Asso exhibited effective insecticidal and repellent activities against red flour beetle, *Tribolium castaneum* (Herbst) (Khani and Asghari 2012; Chaieb et al. 2018). Essential oils of *Tagetes erecta* L. and *Achillea millefolium* L. also showed efficiency as killing and repelling agents for the lesser grain borer, *Rhyzopertha dominica* (F.) (Alkan 2020; Matintarangson 2018). *Tridax procumbens* L. (Asteraceae) is a common weed and pest plant found in tropical and subtropical environments. It is known for its antimicrobial (Jindal and Kumar 2012), antiviral, antibiotic, anti-inflammatory, and insecticidal activities (Sharma and Kumar 2009; Sneha and Ruchi 2010). The study reported herein investigated the fumigant insecticidal activity of *T. procumbens* essential oil against *S. zeamais* and also its effect on seed germination of three Thai varieties of rice, *O. sativa*. Study results could define the potential of extracts from *T. procumbens* as an environmentally friendly control tactic for insect pests in stored grain products.

## Materials and Methods

**Insect culture.** Adults of *S. zeamais* were collected from stored grains in Kantharawichai district, Maha Sarakham Province, Thailand. They were reared in a round plastic container (30 cm diameter, 50 cm height) with 1 kg of maize, *Zea mays* L. (Cyperales: Poaceae), kernels that were pre-frozen for 12 h to kill any attached pests. The containers were maintained at  $30 \pm 5^\circ\text{C}$  and  $70 \pm 5\%$  relative humidity with a 12:12 h light:dark cycle at the Department of Agricultural Technology, Faculty of Technology, Mahasarakham University, Maha Sarakham, Thailand. The insects were allowed to mate and oviposit. Twenty pairs of *S. zeamais* adults were then separated into a new container and fed with 250 g of maize kernels for 5 d. The adults were removed and the newly emerged progeny (aged 7 d) were used for further testing.

**Essential oil extraction.** The aerial parts of *T. procumbens* were collected from local areas of Mahasarakham University, Kantharawichai district, Maha Sarakham Province, Thailand. Fresh samples were dried at  $30^\circ\text{C}$  for 3 d in a hot air oven. The dried aerial parts were ground into powder using an electric blender and sieved through 0.5 mm mesh size. Powdered samples were extracted by the water distillation method with a modified Clevenger-type apparatus. Briefly, the process involved 300 g of powder and 1,000 ml of distilled water, with distillation for 6 h. The essential oil was separated from the remaining water after extraction by centrifugation at 8,000 rpm for 10 min. Pure essential oil was kept in a sealed amber glass bottle and refrigerated at  $4^\circ\text{C}$  in the dark until required for chemical composition analysis and bioassay.

**Chemical composition analysis.** *Tridax procumbens* essential oil from the plant aerial parts was analyzed for chemical composition following the method of Satongrod and Wanna (2020) using Gas Chromatograph-Mass Spectrometer (GC-MS) series Clarus 680 (PerkinElmer, Akron, OH) with an Rtx-5MS capillary column (5% phenyl-methyl polysiloxane stationary phase,  $30 \times 0.32$  mm,  $1.0 \mu\text{m}$  film

thickness). One microliter of sample was 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 and the injector temperature was maintained at 280°C. The initial oven temperature of 45°C was held for 5 min, then increased to 200°C at a rate of 10°C/min and held for 5 min, functioning in electron impact mode of 70eV. A mass analyzer was used as a quadrupole, and the temperature detector was set at 250°C. Spectra were scanned (m/z) from 40 to 1,000 amu. Identification of essential oil components was confirmed by comparing their mass spectra with sample data kept in the National Institute of Standards and Technology (NIST) Mass Spectral Search Program (Gaithersburg, MD) and the ChemStation Wiley Spectral Library (Scientific Instrument Services, Palmer, MA) at a quality match >80%. Chemical composition data of *T. procumbens* essential oil were diagnosed by reading the retention time and percent Area.

**Fumigation toxicity.** The fumigation activity of *T. procumbens* essential oil was evaluated for toxicity and efficiency using the vapor-phase test following the methods of Wanna et al. (2021) at  $30 \pm 5^\circ\text{C}$  and  $70 \pm 5\%$  relative humidity with 12:12 h light:dark cycle. A completely randomized design (CRD) was conducted with four replications. Solutions of *T. procumbens* essential oil were prepared at concentrations of 50, 100, 150, 200, and 250  $\mu\text{l/L}$  air by using hexane. An aliquot (100  $\mu\text{l}$ ) of each concentration was dropped on a Whatman (no. 1) filter paper strip (1.5 cm width, 5.0 cm length) and allowed to evaporate for 2 min at room temperature. Each filter paper strip was suspended in a small glass vial (2.5 cm diameter, 5.0 cm height) from the center of a fumigation bottle screw cap (5.5 cm diameter, 10.5 cm height) to avoid contact between the insects and the filter paper strip. Five pairs of 7-d-old *S. zeamais* adults were released into a fumigation bottle containing 50 g of KDML brown rice (*O. sativa*) grains, and the cap was screwed tightly shut. The control was hexane solvent alone. Numbers of dead *S. zeamais* adults were observed and recorded at 1 and 24 h after treatment. The insects were considered to be dead when no leg or antennae movements were detected. Fumigation efficiency of *T. procumbens* essential oil against *S. zeamais* was evaluated for percentage adult mortality by the following equation: % adult mortality =  $(\text{Nd} - \text{Nt}) \times 100$ , where Nd is the dead number of *S. zeamais* adults and Nt is the total number of *S. zeamais* adults used in the bioassay.

**Effect on newly emerged adult progeny (F1).** Numbers of deaths of *S. zeamais* adults were counted in previous fumigation toxicity experiments of *T. procumbens* essential oil following the method of Wanna et al. (2021). KDML brown rice grains were separated from the remaining surviving adults of *S. zeamais* using a sieve. Bottles containing only grains were stored under the same experimental conditions until the adult progeny (F1) of *S. zeamais* emerged. Numbers of newly emerged adult progeny were observed and recorded every 24 h until no new adult progeny emerged. Based on the life cycle of the control, the counting period of the newly emerged adult progeny of *S. zeamais* was established to avoid generation overlap. Percentage reduction of the newly emerged adult progeny or inhibition rate (IR, percent) was calculated by the equation:  $\%IR = (\text{Cn} - \text{Tn}) \times 100 / \text{Cn}$ , where Cn is the number of newly emerged adult progeny of *S. zeamais* in the untreated (control) grain and Tn is the number of newly emerged adult progeny of *S. zeamais* in the grain treatment.

**Table 1. The five main chemical compounds of *T. procumbens* essential oil.**

Compounds	Retention Time	% Area
Hexanal	3.422	5.34
1,6-dimethylhepta-1,3,5-triene	4.200	1.67
4-heptenal	5.294	4.92
2,4-nonadienal	7.745	3.10
1,2-cyclooctanediol	7.905	11.49

**Effect on seed germination.** The effect of *T. procumbens* essential oil on seed germination was assessed for three varieties of rice (*O. sativa*) seeds commonly grown in Northeastern Thailand as two glutinous rice varieties (RD6 and Kham Na Sinuan) and KDML brown rice. Each treatment evaluated the effectiveness of fumigation toxicity to kill *S. zeamais* adults without release. The experiment was conducted under a CRD with four replications at  $30 \pm 5^\circ\text{C}$  and  $70 \pm 5\%$  relative humidity, with 12:12 h light:dark cycle. Rice seeds were tested using the seed germination method. Seed germination count or percentage of germination were observed for 100 seeds of each plant after 5 d.

**Statistical analyses.** Adult mortality was calculated and adjusted using Abbott's formula (Abbott 1925) when mortality in the control ranged between 5 and 20%. Fumigation toxicity of *T. procumbens* essential oil on *S. zeamais* adults was assessed for dose–mortality response using probit analysis (Finney 1971), and the  $\text{LC}_{50}$  and  $\text{LC}_{95}$  values were determined. Data were analyzed using the *F*-test by one-way analysis of variance, with means compared using the least significant difference test (LSD) at 0.05 probability level ( $P \leq 0.05$ ). All statistical analyses were performed using Statistix, version 9.0 (Analytical Software, Tallahassee, FL).

## Results

**Chemical composition of *T. procumbens* essential oil.** Chemical compositions of essential oil from the aerial parts of *T. procumbens* were analyzed by GC-MS and spectrally assessed against the NIST mass spectral search program and ChemStation Wiley spectral library comparison for mass spectra quality match  $>80\%$ . A total of 25 compounds were identified in *T. procumbens* essential oil, with five main components (26.52%) having Percent Area greater than 1% based on the highest peak, and classified using the International Union of Pure and Applied Chemistry system. The five highest Percent Area compounds were 1,2-cyclooctanediol (11.49%), hexanal (5.34%), 4-heptenal (4.92%), 2,4-nonadienal (3.10%), and 1,6-dimethylhepta-1,3,5-triene (1.67%) (Table 1).

**Fumigation toxicity.** The fumigation toxicity assessment ( $\text{LC}_{50}$  and  $\text{LC}_{95}$ ) of *T. procumbens* essential oil on adults of *S. zeamais* is shown in Table 2. At 1 h, fumigation toxicity to adults of *S. zeamais* in KDML brown rice with essential oil of *T. procumbens* could not be analyzed for  $\text{LC}_{50}$  and  $\text{LC}_{95}$  because death of *S. zeamais* adults was not detected. At 24 h, fumigation toxicity of *S. zeamais* adults by *T.*

**Table 2. Fumigation toxicity of *T. procumbens* essential oil against *S. zeamais* adults at 24 h.\***

<i>n</i>	LC <sub>50</sub> (μ/L air) (95% CL)	LC <sub>95</sub> (μ/L air) (95% CL)	Linear Equation <i>y</i> = <i>ax</i> + <i>b</i>	<i>r</i> <sup>2</sup>
240	1,509.79 (1,434.26–1,585.78)	2,821.74 (2,680.65–2,962.83)	<i>y</i> = 0.0343 <i>x</i> –1.7857	0.6857

\* *n* = number of tested insects (six concentrations, four replications of 40 insects each); LC = lethal concentration (μ/L air), CL = confidence limit; *r*<sup>2</sup> = correlation coefficient.

*procumbens* essential oil for LC<sub>50</sub> was 1,509.79 μ/L air and LC<sub>95</sub> was 2,821.74 μ/L air. Fumigation efficacy of *T. procumbens* essential oil against adults of *S. zeamais* was evaluated, with results shown in Table 3. At 1 h, no deaths of *S. zeamais* adults were recorded in KDML brown rice fumigated with essential oil from *T. procumbens*, and data could not be analyzed. At 24 h, adult mortality of *S. zeamais* by fumigating KDML brown rice with *T. procumbens* essential oil was significantly different ( $F = 3.05$ ;  $df = 5, 18$ ;  $P = 0.0362$ ). Concentrations of 50–200 μ/L air were not significantly different ( $P > 0.05$ ) for adult mortality of *S. zeamais* ( $0.00 \pm 0.00\%$  to  $2.50 \pm 5.00\%$ ). The highest concentration of 250 μ/L air was significantly different ( $P \leq 0.05$ ) compared with the other concentrations, with adult mortality of  $10.00 \pm 8.16\%$ .

**Effect on adult F1 progeny.** The effects of *T. procumbens* essential oil on newly emerged adult progeny (F1) of *S. zeamais* and its inhibition rate were measured after fumigation testing for 14 d. Numbers of adult F1 progeny of *S. zeamais* and their inhibition rates are shown in Table 4. *Tridax procumbens* essential oil affected newly emerged adult progeny of *S. zeamais* with the highest significant difference ( $F = 62.17$ ;  $df = 5, 18$ ;  $P = 0.0000$ ). The highest concentration at 250 μ/L air yielded number of adult F1 progeny of *S. zeamais* as  $11.75 \pm 5.56$  and was significantly different ( $P <$

**Table 3. Adult mortality (Mean ± SE) of *S. zeamais* in Jasmine brown rice fumigated with essential oil from *T. procumbens*.\***

Concentration (μ/L air)	Mean ± SE Adult Mortality (%) of <i>S. zeamais</i>	
	1 h	24 h
50	0.00 ± 0.00	0.00 ± 0.00b
100	0.00 ± 0.00	0.00 ± 0.00b
150	0.00 ± 0.00	2.50 ± 5.00b
200	0.00 ± 0.00	2.50 ± 5.00b
250	0.00 ± 0.00	10.00 ± 8.16a
<i>F</i> -test	N/A	**

\* N/A represents not applicable. Means within the same column followed by the same letter are not significantly different (LSD:  $P > 0.05$ ).

\*\* Represents significant difference at  $P \leq 0.05$ .

**Table 4. Number of adult F1 progeny and percentage of adult F1 progeny inhibition of *S. zeamais* exposed to *T. procumbens* essential oil.\***

Concentration ( $\mu\text{L}$ /L air)	Number of Adult F1 Progeny (mean $\pm$ SE)	% Inhibition Rate (IR)
0 (control)	164.75 $\pm$ 16.05a	—
50	102.00 $\pm$ 16.08b	38.09
100	71.75 $\pm$ 16.58c	56.45
150	53.00 $\pm$ 18.89c	67.83
200	24.00 $\pm$ 4.69d	85.43
250	11.75 $\pm$ 5.56e	92.87
<i>F</i> -test	**	—

\* Means within the same column followed by the same letter are not significantly different (LSD:  $P > 0.05$ ); — represents not applicable.

\*\* Represents significant difference at  $P \leq 0.01$ .

0.05) compared to the other concentrations. The concentration of 200  $\mu\text{L}$ /L air yielded 24.00  $\pm$  4.69 adults. All concentrations of *T. procumbens* essential oil yielded number of adult F1 progeny that were significantly less ( $P < 0.05$ ) than the control as 0  $\mu\text{L}$ /L (164.75  $\pm$  16.05 adults). The concentration of *T. procumbens* essential oil at 250  $\mu\text{L}$ /L air showed the highest inhibition rate of newly emerged adult F1 progeny of *S. zeamais* at 92.87%, while concentrations of 200, 150, and 100  $\mu\text{L}$ /L air yielded inhibition rates of more than 50% at 85.43, 67.83, and 56.45%, respectively.

**Effect on seed germination.** Effects of 6 concentrations of *T. procumbens* essential oil on seed germination of three varieties of Thai rice (RD6, Kham Na Sinuan, and KDML) were evaluated after testing for 14 d by the fumigation method. Germination percentages after testing at 5 d are shown in Table 5. Significantly highest seed germination was found in RD6 ( $F = 4.68$ ;  $df = 5, 18$ ;  $P = 0.0065$ ), with significantly different effects on seed germination of KDML ( $F = 3.73$ ;  $df = 5, 18$ ;  $P = 0.0171$ ). There was no significant difference with the Kham Na Sinuan ( $F = 1.90$ ;  $df = 5, 18$ ;  $P = 0.1435$ ). The concentration of 250  $\mu\text{L}$ /L air impacted seed germination of all three rice varieties compared with the control. Seeds of Kham Na Sinuan were slightly affected by *T. procumbens* essential oil, with germination percentage at 250  $\mu\text{L}$ /L air decreased slightly to 96.00  $\pm$  2.83%, but not significantly different compared to the control (98.75  $\pm$  0.50%). By contrast, germination percentage of RD6 (96.75  $\pm$  0.96%) and KDML (96.00  $\pm$  1.41%) at 150  $\mu\text{L}$ /L air were not significantly different compared to the control (98.00%). The highest concentration of 250  $\mu\text{L}$ /L air obtained from the seed germination test of the three rice varieties with *T. procumbens* essential oil yielded percentages of seed germination greater than 90.00%.

## Discussion

For chemical composition analysis, *T. procumbens* essential oil from the aerial parts was obtained by water distillation and analyzed by gas chromatography

**Table 5. Germination percentage (mean  $\pm$  SE) of the three Thai rice seed varieties exposed to *T. procumbens* essential oil by fumigation method.**

Concentration ( $\mu\text{L/L}$ air)	RD6	Kham Na Sinuan	KDML
0 (control)	98.00 $\pm$ 1.63a <sup>a</sup>	98.75 $\pm$ 0.50	98.00 $\pm$ 0.82a
50	98.00 $\pm$ 0.82a	98.50 $\pm$ 0.58	97.50 $\pm$ 1.29ab
100	98.00 $\pm$ 1.41a	98.00 $\pm$ 1.41	96.75 $\pm$ 1.26ab
150	96.75 $\pm$ 0.96ab	97.75 $\pm$ 0.50	96.00 $\pm$ 1.41ab
200	95.50 $\pm$ 1.29bc	97.25 $\pm$ 1.26	95.25 $\pm$ 2.87bc
250	94.25 $\pm$ 2.22c	96.00 $\pm$ 2.83	93.25 $\pm$ 2.22c
<i>F</i> -test	**	ns <sup>b</sup>	*

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

<sup>b</sup> ns represents not a significant difference at  $P > 0.05$ .

\* Represents a significant difference at  $P \leq 0.05$ .

\*\* Represents a significant difference at  $P \leq 0.01$ .

coupled with mass spectrometry (GC-MS). The principal compounds were 1,2-cyclooctanediol, hexanal, 4-heptenal, 2,4-nonadienal, and 1,6-dimethylhepta-1,3,5-triene. Our results differed from those of Joshi and Badakar (2012), who reported on essential oil of the flowers of *T. procumbens* obtained by hydro-distillation and analyzed by gas chromatography equipped with a flame ionization detector (GC-FID) and GC-MS. They reported the most abundant compounds as (Z)-falcarinol,  $\alpha$ -selinene, limonene, zerumbone, linalool,  $\beta$ -pinene oxide,  $\alpha$ -terpineol, methyl chavicol, (E)-anethole, humulene epoxide II, (Z)- $\beta$ -curcumen-12-ol, cyclopentadecanolide, and n-tricosane. Likewise, Manjamalai et al. (2012) reported that the essential oil of the leaves of *T. procumbens* L. contained 14 compounds, namely  $\alpha$ -pinene, 1,3,6-octatriene, camphene,  $\beta$ -pinene, sabinene, phellandrene, L-limonene,  $\beta$ -ocimene, trans- $\beta$ -ocimene, trans-caryophyllene,  $\gamma$ -elemene, spathulenol, torreyol, and aromadendrene. Our results disagreed with Brandão et al. (2021), who indicated that GC-MS analysis of the essential oil of *T. procumbens* identified 20 compounds divided between sesquiterpenes and oxygenated terpenes. Among the major compounds of *T. procumbens* essential oil, thymol,  $\gamma$ -terpinene, and  $\sigma$ -cymene are the most important. Different production regions give diverse compound concentrations of essential oils according to genetic and physiological factors as well as climatic conditions, type of soil, and extraction technique (Cansian et al. 2008).

Killing toxicity of *T. procumbens* essential oil products against *S. zeamais* adults was very low, with high a LC<sub>50</sub> value greater than 1,500  $\mu\text{L/L}$  air at 24 h. This result contradicted Kordali et al. (2013), who found that essential oils from Asteraceae, such as *Achillea biserrata* M. Bieb., *Achillea wilhelmsii* C. Koch, *Achillea coarctata* Poiret, and *Artemisia santonicum* L. were highly toxic to *S. zeamais* adults with a low LC<sub>50</sub> value of less than 2.5  $\mu\text{L/L}$  air at 96 h. Matintaranson (2018) found that essential oils from Asteraceae, including marigold (*Tagetes erecta* L.), gave high

fumigation and exposure to *Rhizopertha dominica*, with LC<sub>50</sub> toxicity values of 25 µl/L air and 38.45 µl/L air at 24 h, respectively. *Tridax erecta* essential oil at a concentration of 50 µl/L air affected the mortality of *Rhizopertha dominica* and was effective as a repellent and fumigant.

Using essential oils from plants is an alternative storage pest control that is safe for humans and animals and does not endanger the environment (Koul et al. 2008). Essential oils are secondary metabolites with constituents capable of killing bacteria, fungi, parasites, and insects (Bakkali et al. 2008). Radha and Susheela (2014) explained that plant secondary metabolites enter the insect body through the airway, orally, and leg joints. This affects cellular respiration of insects by inhibiting or hampering NADH-co-enzyme ubiquinone reductase (complex I) and the electron transport system in mitochondria. As a result, the insects lack oxygen and eventually die. Aref and Valizadegan (2015) explained that the pungent odor of plant extracts affects insect behavior. The head, tentacles, and mouth appendages have chemical sensory neurons (chemosensilla). If the odor is pungent or inappropriate, the central nervous system directs the chemical sensory neurons to stop the response and retreat.

In this study, *T. procumbens* was tested as an essential oil and found to have a killing effect on *S. zeamais* adults. The toxicity of essential oils varies according to the amount, concentration of the plant product, and duration of exposure. Nenaah (2014) reported that essential oils of the three species, *Achillea biebersteinii*, *Achillea fragrantissima*, and *Ageratum conyzoides* had a mortality effect against *Sitophilus oryzae*, *Rhizopertha dominica*, and *Tribolium castaneum*. The toxicity of essential oils varies according to the concentration of the plant, the pests tested, and the duration of exposure. Differences in responses of the test insects may be due to differences in morphology, physiology, and behavior between insect species.

Essential oil from *T. procumbens* in this study reduced the germination percentage of RD6, Kham Na Sinuan, and KDML rice varieties, but the germination percentage obtained was still higher than 90%. All three types of Thai rice seeds retained good germination quality. However, greater reduction in germination percentages resulted when using higher concentrations of the essential oil. In the tailbone, an inhibitor known as allelopathy inhibits the germination and growth of rice seeds. In conclusion, the essential oil from *T. procumbens* reduced *S. zeamais* populations, but it may not be suitable for use in stored grain pest management because of its impact to grain germination.

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