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Fumigant toxicity and bioactivity *of Wedelia trilobata* essential oil against cowpea weevil (*Callosobruchus maculatus*)

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Abstract Cowpea weevil, *Callosobruchus maculatus* (F.), is a storage product pest mainly controlled using synthetic insecticides. Bioactive plant essential oils can provide safer, less environmentally destructive control options. Chemical composition analysis of *Wedelia trilobata* essential oil was found alpha-pinene (34.96%) as the major component. Four chemical compounds: alpha-pinene, p-cymene, linalool and camphene were effective against adults of *C. maculatus*. The essential oil of *W. trilobata* was fumigant toxic at the LC_{50} to adults of *C. maculatus* with mortality at 24 h using 5304.61 µL/L air, 48 h for 2813.59 µL/L air, and 72 h for 2123.76 µL/L air. *W. trilobata* essential oil at a concentration of 1000 µL/L air gave optimal inhibitory effect on oviposition and adult F1 progeny emergence of *C. maculatus* at 73.08% and 89.13%, respectively.

Keywords: Asteraceae, Chemical composition, Toxicity, Essential oils, Stored product pests

Introduction

Mung bean, *Vigna radiata* (L.) R. Wilczek, is an important economic crop in Thailand for daily human consumption and as an export commodity to foreign countries. Mung bean is rich in vitamin E as an antioxidant and a source of protein, with high carbohydrate content. All parts of mung bean can be processed and used in a variety of ways such as bean sprouts, mung bean flour, vermicelli and making various desserts (Agricultural Research Development Agency (Public Organization), 2016). In 2019, Thailand had 803,522 rai (128,563 ha) of mung bean plants, with an average yield of 115 kg per rai (719 kg per ha) and total output of 92,472 tons against a demand of 113,291 tons. A total of 16,753 tons was exported, while 26,671 tons were imported (Bureau of Agricultural Commodities Promotion and Management, 2019). Mung bean production is hampered by outbreaks of infestation from stored insect pests while seeds are stockpiled for consumption or distribution. Insect pests reduce

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seed quality, resulting to lower value. The key pest of mung beans is the cowpea weevil, *Callosobruchus maculatus* (F.). This insect hatches and grows inside the mung bean seed until it becomes an adult, causing damage such as reduced seed or grain weight, low germination rate and loss of nutritional value (Melo *et al*., 2010; Oke and Akintunde, 2013).

Several methods are being used to combat *C. maculatus* infestation but the most common is application of synthetic fumigants such as phosphine. The fumigant penetrates through the seed pile and effectively eliminates postharvest insect pests at all growth stages (Liu and Liu, 2014). However, the use of fumigants in postharvest insect pest control adversely impacts to animals and the environment. Over time, insects build up resistance to synthetic chemicals (Pimentel *et al*., 2007). Reports on the lesser grain borer *Rhyzopertha dominica* (Fabricius) showed resistance to phosphine (Song *et al*., 2011), while using chemicals may also affect human health and be harmful to consumers when improperly applied. Therefore, utilizing extracts and essential oils from local plants as fumigants to reduce the use of harmful chemicals has attracted increasing research interest. Essential oils are highly toxic to insect pests that destroy grain but show very slight toxicity to warm-blooded animals. The active ingredients of plant essential oils are not durable and easily decompose, thereby negating the accumulation of toxic substances with zero environmental impact (Isman, 2000; Bakkali *et al*., 2008).

Essential oils are secondary metabolites produced by plants and are present in various parts of the plant such as leaves, seeds, fruit, flowers and bark. Each essential oil is composed of different complex ingredients. Some of the secondary substances in plant essential oils have properties that can kill various insect pests as repellents, insecticides, antifeedant, oviposition deterrent and growth regulators (Koul *et al*., 2008; Mahmoudvand *et al*., 2011; Wanna and Khangkhun, 2018; Wanna and Kwang-Ngoen, 2019; Wanna, 2021). Many studies have demonstrated the efficacy of essential oils from various plant families that can be used to prevent *C. maculatus* (F.) such as the Zingiberaceae including white angle (*Curcuma cochinchinensis* Gagnep.), turmeric (*Curcuma longa* L.), ginger (*Zingiber officinale* Roscoe); the Rutaceae including kaffir lime (*Citrus hystrix* DC.), curry (*Murraya Koenigii* (L.) Spreng.), Indian prickly ash (*Zanthoxylum limonella* (Dennst.) Alston.); the Piperaceae including black pepper (*Piper nigrum* L.), long pepper (*Piper hispidinervum* DC.); the Lamiaceae including Indian borage (*Plectranthus amboincus* (Lour.) Spreng), kitchen mint (*Mentha cordifolia* Opiz.), holy basil (*Ocimum sanctum* L.), anise hyssop (*Agastache foeniculum* (Pursh) Kuntze), summer savory (*Satureja hortensis* L.); the Apiaceae including coriander (*Coriandrum sativum* L.), celery (*Apium graveolens* L.), fennel (*Foeniculum vulgare* Mill.), dill

(*Anethum graveolens* L.), cumin (*Cuminum cyminum* L.); the Myrtaceae including clove (*Syzygium aromaticum* (L.) Merrill & Perry), lemonscented gum (*Eucalyptus citriodora* Hook); the Gramineae including lemon grass (*Cymbopogon citratus* (DC.) Stapf), java citronella (*Cymbopogon winterianus* Jowitt); the Lauraceae including cinnamon (*Cinnamomum zeylanicum* Blume), and the Euphorbiaceae including physic nut (*Jatropha curcas* L.), castor bean *Ricinus communis* L. (Krishnappa *et al*., 2011; Ebadollahi *et al*., 2012; Gusmao *et al*., 2013; Oliveira *et al*., 2017; Wanna, 2017; Wanna and Choathsri, 2017; Matintarangson, 2018b; Satongrod and Wanna, 2020; Wanna, 2021).

Asteraceae is another very interesting plant family, with reports detailing effectiveness in the prevention of various postharvest insect pests. Billygoat weed (*Ageratum conyzoides* L.), Siam weed (*Chromolaena odorata* (L.) King & Robinson), and lantana (*Lantana camara* L.) showed efficacy in killing maize weevil, *Sitophilus zeamais* Motschulsky (Bouda *et al*., 2001). Essential oil of Mexican marigold (*Tagetes erecta* L.) proved effective as a repellant and lethal toxicity agent to *R. dominica* (Matintarangson, 2018a), while essential oil from allheal (*Achillea wilhelmsii* C. Koch) and false fleabane (*Pulicaria gnaphalodes* Ventenat) were effective as a killing agent for *C. maculatus* (Khani and Asghari, 2012). Essential oil from Judean wormwood (*Artemisia judaica* L.) was effective in reducing the fecundity and adult emergence of offspring (F1) of *C. maculatus* (Abd-Elhady, 2012).

Wedelia trilobata (L.), commonly called climbing wedelia, is a dwarf shrub and a member of the Asteraceae (formerly Compositae), the sunflower or daisy family. Wedelia is an invasive weed that grows in wide-ranging tropical and subtropical areas (IUCN, 2001), and is well known for its natural properties in Thai traditional medicine, with reports of several types of biological activities. Anti-insect properties of the plant involve toxic effects against many insects (Yooboon *et al.*, 2019). Earlier studies discovered the insecticidal activities of *W. trilobata* extracts, while Khater (2009) reported that plant parts of fruits, leaves and stem extracts were effective against *Synthesomyia nudiseta* larvae. *W. trilobata* essential oil was also effective in fumigation and contact toxicity exposure to adults of the red flour beetle *Tribolium castaneum* Hbst., *S. zeamais* and southern cowpea weevil *C. chinensis* (L.) (Cheng *et al*., 2014; Khater and Shafeiy, 2015; Wanna *et al.*, 2021). *W. trilobata* crude extracts showed larvicidal effect on *Spodoptera litura*, *S. exigua* and *Plutella xylostella* larvae after topical application (Junhirun *et al*., 2018). A recent study reported that *W. trilobata* powder and essential oil showed efficacy in protection against *S. zeamais.* Here, the chemical composition of *W. trilobata* essential oil from fresh leaves was investigated, and fumigation toxicity was evaluated against

antiovipositional activity and adult F1 progeny production against the cowpea weevil *C. maculatus* in the laboratory.

Materials and methods

Insect culture

C. maculatus specimens were reared following the method of Satongrod and Wanna (2020). Adults were obtained from mung bean seed stored in sub-district Khok Phet Phatthana, district Bamnet Narong, Chaiyaphum Province, Thailand. The insects were reared to increase population numbers at the Department of Agricultural Technology, Faculty of Technology, Mahasarakham University, and cultured under laboratory conditions at 30±5 ℃, 70±5 %RH and 8h:16h (light: dark) photoperiod. One kilogram of mung bean (*Vigna radiata* (L.) Wilczek) was used as the food medium. Fifteen adult pairs were selected and released in a cylindrical plastic container (22 cm diameter, 30 cm height) for 7-10 days for mating and oviposition. The *C. maculatus* adults were then sieved and removed. Mung beans with a covering of eggs on the seed surface were separated into a cylindrical plastic container (11 cm diameter, 11 cm height). After 20 days, new adults of *C. maculatus* emerged. Five-day-old specimens were used for all bioassays.

Essential oil extraction

Essential oil extraction was modified by following Satongrod and Wanna (2020). Extraction of essential oil was performed using the water distillation method. Plant parts were taken from the 4th, 5th and 6th fresh leaf pairs of *W. trilobata*, washed and shredded into small pieces in a 2,000 mL round glass vial, and 1,500 mL of distilled water was added. A round glass vial was used for the water distillation process lasting 3 h at 100-120 ℃. All the plant parts were immersed in the boiling water. Essential oil of *W. trilobata* was released during the distillation and purified by centrifuging at 10000 rpm for 10 min to remove the remaining water. The oil was kept in an amber bottle in a fridge at 4 ℃ until required for further bioassays.

Identification of chemical compositions

The chemical composition of the essential oil was investigated following the method of Satongrod and Wanna (2020). Chemical compounds contained in *W. trilobata* essential oil from fresh leaves were analyzed using 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). One microliter (100,000 ppm) 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 ℃. Initial oven temperature was 45 ℃ for 5 min, then increased to 200 ℃ at a rate of 10 ℃/min and held for 5 min, functioning in electron impact mode of 70 eV. A mass analyzer was used as a quadrupole, and the temperature detector was set at 250 ℃. Spectra were scanned (m/z) from 40 to 1000 amu. Identification of essential oil components was assumed by comparing their mass spectra with those kept in the National Institute of Standards and Technology (NIST) Mass Spectral Search Program and the ChemStation Wiley Spectral Library. Essential oil constituents were determined by comparing the substances with mass spectra of substances with quality match more than 80%. Chemical composition data of *W. trilobata* essential oil from fresh leaves were diagnosed by reading the retention time and %area.

Fumigation toxicity

Fumigation toxicity of *W. trilobata* essential oil on *C. maculatus* adults was tested as previously described by Satongrod and Wanna (2020). A sealed fumigation bottle (5.5 cm diameter, 10.5 cm height) was used for bioassay vapor-phase testing. Four replications with six concentrations were tested under laboratory condition of 30±5 ℃, 70±5 %RH and 8h:16h (light: dark) photoperiod. Essential oil solutions were diluted with 100% acetone in concentrations of 0, 600, 1200, 1800, 2400 and 3000 µL/L air. One hundred microliters of each essential oil solution were dropped on a small square of filter paper (1.5 cm width, 5 cm length) and evaporated at ambient temperature for 2 min. A control was prepared using 100% acetone with 100 µL. A filter paper was placed in a small glass vial (2.5 cm diameter, 5 cm height) and hung from the center of the cover of a fumigation bottle (5.5 cm diameter, 10.5 cm height). The essential oil vapor diffused inside the fumigation bottle. Ten pairs of *C. maculatus* adults (5 days old) were released in a fumigation bottle and the cover was firmly closed. The number of dead *C. maculatus* adults was recorded at 24, 48 and 72 h after exposure. Percentage of adult mortality was calculated using the formula [(NC/NT)]x100, where NC represented the dead number of *C. maculatus* adults and NT represented the total number of *C. maculatus* adults used in the bioassay. If the value of adult mortality in the control was in the range 5-20%, adult mortality value in each treatment was adjusted by

Abbott's formula (Abbott, 1925). The LC_{50} (lethal concentration) value of fumigation toxicity of *W. trilobata* essential oil from fresh leaves on *C. maculatus* adults was use to analyze the dose-mortality response by using probit analysis.

Oviposition inhibition

Oviposition inhibitory activity of *W. trilobata* essential oil on *C. maculatus* was tested by the vapor-phase method in a sealed fumigation bottle (5.5 cm diameter, 10.5 cm height). Four replications with six concentrations were tested under laboratory condition of 30±5 ℃, 70±5 %RH and 8h:16h (light: dark) photoperiod. Solutions of *W. trilobata* essential oil were prepared at concentrations of 0, 200, 400, 600, 800 and 1000 µL/L air with 100% acetone. Exactly 100 µL of each *W. trilobata* essential oil solution was dropped on a small square of filter paper (1.5 cm width, 5 cm length) and evaporated for 2 min at ambient temperature. A control was arranged with 100 µL of 100% acetone. A filter paper was placed in a small glass vial (2.5 cm diameter, 5 cm height) and hung from the middle of the lid of a fumigation bottle (5.5 cm diameter, 10.5 cm height). Vapor of the essential oil diffused inside the fumigation bottle. A total of 240 female adults (5 days old) of *C. maculatus* were released into a fumigation bottle that was then tightly closed for 7 days. A female adult of *C. maculatus* that had been fumigated with *W. trilobata* essential oil solution, was mated with a male adult of *C. maculatus* that had not been fumigated with *W. trilobata* essential oil solution (1 pair of *C. maculatus* adults per replication) into a glass bottle (5.5 cm diameter, 10.5 cm height) containing 10 g of new mung bean seeds. Adults of *C. maculatus* were separated from the glass bottle after mating and allowing to lay eggs for 3 days. The number of eggs of *C. maculatus* on the mung bean seed surface was counted and recorded. Percentage of oviposition inhibition of *C. maculatus* was calculated using the formula [(NC-NT)/NC]x100, where NC represented the egg number in the control, and NT was the egg number in the essential oil treatment. Data were analyzed for the F-test statistic by one-way analysis of variance (ANOVA) and means were compared using Duncan's Multiple Range Test (DMRT) at the 0.05 probability level ($p < 0.05$).

Adult F1 progeny emerged inhibition

The adult F1 progeny inhibition bioassay of *C. maculatus* was carried out following the oviposition inhibitory activity bioassay. After mating and oviposition for 3 days, female adults of *C. maculatus* were separated from the

glass bottle. Mung bean seeds with eggs on the seed surface were selected, placed in the original bottle, and kept under laboratory condition at 30±5 ℃, 70±5 %RH and 8h:16h (light: dark) photoperiod. After 20 days, numbers of adult F1 progeny emergence of *C. maculatus* were counted and recorded. Percentage of adult F1 progeny emergence inhibition of *C. maculatus* was calculated using the formula [(NC-NT)/NC]x100, where NC represented the total number of adult F1 progeny emergence in the control, and NT was the number of adult F1 progeny emergence in the essential oil treatment. Data were analyzed for the F-test statistic by one-way analysis of variance (ANOVA) and means were compared using Duncan's Multiple Range Test (DMRT) at the 0.05 probability level ($p < 0.05$).

Results

Compositions of W. trilobata essential oil

Analysis of *W. trilobata* essential oil from fresh leaves identified 30 major chemical constituents (Table 1). Alpha-pinene (34.96%) was the major component followed by alpha-phellandrene (12.73%), germacrene D (12.12%), d-limonene (4.48%), alpha-myrcene (4.41%), bicyclogermacrene (4.28%), caryophyllene (3.15%), cedrene (2.91%), humulene (2.39%), junenol (1.96%), spathulenol (1.82%), beta-pinene (1.54%), p-cymene (1.41%) and isoledene (1.16%). Other constituents were recorded at less than 1%.

Fumigation toxicity

The concentration of *W. trilobata* essential oil that resulted to death of *C. maculatus* adults was determined as the LC_{50} value, with fumigation toxicity inferred by confidence limits (Table 2). Essential oil of *W. trilobata* showed highest LC_{50} value and lowest toxicity ratios for the lethal concentration. A linear regression model gave the best fit for results of adult mortality, indicating that LC_{50} value and toxicity were inversely proportional to increasing concentrations of essential oil. Probit analysis showed that *C. maculatus* had low susceptibility to *W. trilobata* essential oil, with LC_{50} values at 24, 48 and 72 h of 5304.61 (2514.03-5768.80), 2813.59 (1301.01-3225.04) and 2123.76 (1098.10-2353.25) µL/L air, respectively. Results of linear regression and probit analysis showed a positive linear relationship between percentage of adult mortality and lethal concentration (LC values) of *W. trilobata* essential oil against *C. maculatus* adult, as indicated by higher values of the regression coefficient (r^2) and graph slope. Higher values of r^2 indicated that efficacy of the essential oil increased as concentration level increased. LC values decreased

when adults of *C. maculatus* were exposed to *W. trilobata* essential oil for a longer period. Results indicated that *W. trilobata* essential oil killed more adults of *C. maculatus* when exposure duration was extended from 24 to 72 h.

Table 1. Chemical compositions of essential oil from *W. trilobata* leaves

No.	Compounds	Retention time	%Area
1	alpha-pinene	5.457	34.96
2	camphene	5.627	0.68
3	beta-terpinene	6.105	0.79
4	alpha-myrcene	6.375	4.41
5	beta-pinene	6.588	1.54
6	alpha-phellandrene	7.348	12.73
7	p-cymene	7.711	1.41
8	D-Limonene	7.986	4.48
9	alpha-ocimene	8.349	0.28
10	alpha-ylangene	21.383	0.13
11	beta-elemene	22.011	0.25
12	caryophyllene	23.424	3.15
13	humulene	24.840	2.39
14	germacrene D	26.232	12.12
15	beta-selinene	26.324	0.27
16	bicyclogermacrene	26.704	4.28
17	isoledene	27.368	1.16
18	isocaryophyllene	29.174	0.42
19	spathulenol	29.687	1.82
20	guaiol	30.383	0.17
21	caryophyllene oxide	30.708	0.12
22	junenol	31.333	1.96
23	cubenol	31.413	0.91
24	beta-eudesmol	32.374	0.12
25	alpha-cadinol	32.554	0.61
26	cedrene	34.111	2.91
27	methyl4,7,10,13,16,19docosahexaenoate	43.631	0.80
28	neophytadiene	47.674	0.80
29	isopimaradiene	51.688	0.44
30	nandrolone phenylpropionate	56.199	0.12
	Unknown		3.77
	Total		**100.00**

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

Time (h)	n	LC_{50} (95% CL)	y = ax + b	r^2
24	480	5304.61 (2514.03-5768.80)	y = 0.0102x - 4.1071	0.79
48	480	2813.59 (1301.01-3225.04)	y = 0.0205x - 7.6786	0.81
72	480	2123.76 (1098.10-2353.25)	y = 0.0287x - 10.952	0.89

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

Oviposition inhibition

Bioassay results for oviposition inhibitors showed a significant difference ($p < 0.05$) in the number of eggs of *C. maculatus* for *W. trilobata* essential oil and the control treatments. Egg number decreased with rising concentrations from 600 to 1000 µL/L air (Table 3). *W. trilobata* essential oil at 1000 µL/L air gave the lowest number of eggs at 5.25 compared to 19.50 in the control. There was no significant difference in egg numbers between concentrations of 600 and 800 µL/L air of *W. trilobata* essential oil at 15.25 and 975, respectively. Percentage of oviposition inhibition of *C. maculatus* female adults on mung bean seed surfaces when allowed to lay eggs for 3 days showed that the highest concentration of 1000 µL/L air gave optimal oviposition inhibition at 73.08% followed by 800 and 600 µL/L air at 50.00% and 21.79%, respectively. On the other hand, concentrations of 0-200 µL/L air of *W. trilobata* essential oil were more active in stimulating female adults of *C. maculatus* for oviposition compared with the control.

Table 3 . Number of egg and oviposition inhibition percentage of *C. maculatus* female adult exposed to *W. trilobata* essential oil

Concentration (µL/L air)	Number of eggs	% oviposition inhibition
control	19.50 ab	-
0	27.25 a	-39.74
200	21.75 ab	-11.54
400	19.75 ab	-1.28
600	15.25 abc	21.79
800	9.75 bc	50.00
1000	5.25 c	73.08
F-test	*	

Means within the same column followed by the same letter are not significantly different at $p < 0.05$ by Duncan's Multiple Range Test (DMRT)

Adult F1 progeny emerged inhibition

Fumigation efficacy of *W. trilobata* essential oil on adult F1 progeny emergence inhibition of *C. macula*tus showed significant differences ($p < 0.05$) after fumigation at all concentrations (Table 4). *W. trilobata* essential oil at a concentration of 1000 µL/L air gave the lowest adult F1 *C. maculatus* progeny emergence with 1.25 adults compared to 12.00 adults in the control. There were no significant differences between concentrations of 400, 600 and 800 µL/L air, with adult F1 numbers of *C. maculatus* at 10.50, 6.25 and 4.50, respectively. The highest efficacy in inhibition of adult F1 progeny emergence of *C. maculatus* was 76.19% when treated with *W. trilobata* essential oil at a concentration of 1000 µL/L air.

Table 4. Number of adult F1 progeny and percentage of adult F1 progeny inhibition of *C. maculatus* exposed to *W. trilobata* essential oil

Concentration (µL/L air)	Number of adult F1 progeny	% adult F1 progeny inhibition
control	12.00 ab	38.46
0	17.50 a	35.78
200	12.75 ab	41.38
400	10.50 abc	46.84
600	6.25 bc	59.02
800	4.50 bc	53.85
1000	1.25 c	76.19
F-test	*	*

Means within the same column followed by the same letter are not significantly different at $p < 0.05$ by Duncan's Multiple Range Test (DMRT).

Discussion

Chemical component analysis of *W. trilobata* essential oil from fresh leaves concurred with previous reports that alpha-pinene was the major constituent. Baisaenga *et al.* (2017) reported alpha-pinene (19.46%) as the main constituent of essential oil from *W. trilobata* leaves, while Khater and Shafeiy (2015) recorded 18.20% as the main component from leaves and stems of *W. trilobata*. Cheng *et al.* (2014) also determined that the principal active ingredient of *W. trilobata* essential oil was alpha-pinene at 28.79%, while Wu and Zhang (2008) and Li *et al*. (2016) recorded the major chemical constituents of *W. trilobata* as *ent*-kaurane diterpenes, sesquiterpene lactones and triterpenes, with a variety of biological activities such as antibacterial, antitumor, hepatoprotective and central nervous system depressant properties. Different quantities and types of chemical constituents in the leaves result from the growing environment, time period of harvesting and extraction method (Ozcan and Chalchat, 2006). Differences in the composition of essential oil were associated with relative amounts of constituents and not the presence/absence of a specific component. Variations of chemical compounds may be qualified to genetics, plant parts, the environment (temperature, photoperiod, and hygrometry) and the harvesting method (Gil *et al.*, 2002; Ortega *et al*., 2011). In this study, some other important chemical constituents such as germacrene D, camphene, beta-pinene, caryophyllene oxide and p-cymene were found. These were also analyzed from Asteraceae family members as *Tithonia diversifolia* (Hemsl) A. Grey., *Chamomilla recutita* L. and *Cosmos bipinnatus* Cav. (Olajuyigbe and Ashafa, 2014; Raala *et al*., 2011; Sousa *et al*., 2018).

Results indicated that the essential oil of *W. trilobata* was highly toxic to adults of *C. maculatus*, concurring with Forouzan *et al*. (2016) and Khani and

Asghari (2012) who reported that essential oils of Asteraceae such as *Pulicaria gnaphalodes, Achillea wilhelmsii* and *Artemisia annua* had fumigation toxicity against many species of stored insect pests including *C. maculatus*, *T. castaneum* and *S. granaries*. Insecticidal activity of the essential oils of many plants has been attributed to monoterpenoids (Tong and Coats, 2010), reported as fumigants and contact toxicants for various insect pests (Rice and Coats, 1994). Major volatile constituents of *W. trilobata* essential oil obtained from leaves were alpha-pinene, germacrene D, d-limonene (Nirmal *et al.*, 2005) and phellandrene (Khater and Shafeiy, 2015).

Results revealed significant oviposition inhibition and moderate inhibition of *C. maculatus* adult F1 progeny emergence in mung bean seed treated with *W. trilobata* essential oil 1000 μL/L air. *W. trilobata* essential oil inhibited oviposition of female adults of *C. maculatus*. Significantly different *C. maculatus* egg numbers resulted from the potency of monoterpene, a key constituent of *W. trilobata* essential oil that reduced the fecundity of *C. maculatus* adults. Regnault-Roger and Hamraoui (1994; 1995) found significant differences in numbers of eggs laid between essential oils and control treatments. Essential oils and monoterpene compounds reduced the fecundity of *Acanthoscelides obtectus*. This study demonstrated that *W. trilobata* essential oil was effective in oviposition inhibition of female adults of *C. maculatus*. Findings were similar to Brari and Thakur (2017) who showed that essential oil from *Artemisia maritima* (Asteraceae) at a concentration of 100 μL/mL impacted inhibition of eggs laid by *C. analis*, the same genus as the cowpea weevil by up to 73.18% and it was reducing the adult F1 emergence of *C. analis* with 81.67%.

Results indicated that *W. trilobata* essential oil showed high potential for efficacy in the control of *C. maculatus* as evidenced by fumigation toxicity, antioviposition and inhibition of adult F1 progeny emergence. These activities are important and desired for the development of new alternative, sustainable products for the safe and effective control of stored product pests under integrated pest management (IPM) programs. Therefore, *W. trilobata* essential oil can be recommend as an effective bioactive insecticide for use in the management of *C. maculatus*.

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