



## Feeding Deterrence and Insecticidal Activity of Selected Plant Essential Oils Against the Larger Grain Borer *Prostephanus truncatus* in Stored Maize Grains

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### ABSTRACT

In our screening program for botanical pesticides from local plants, the powders and essential oils from *Plectranthus marrubiooides* (Hochst ex Benth), *Tetradenia riparia* (Hochst Codd), *Ocimum suave* (Wild), *Lippia javanica* (Burm, Spreng), and *Ocimum lamiifolium* (Hochst ex Benth) were found to possess insecticidal activity against the maize weevil, *Prostephanus truncatus*. The essential oils from aerial parts of the plants were obtained by hydro-distillation and tested under laboratory conditions ( $25 \pm 1^\circ\text{C}$ , 70-75% R.H.) for their ability to protect the grains from damage by *P. truncatus*. The insects were reared and tested on whole maize grain (variety DK 8031). Bioassays were conducted using five dilution levels of essential oils in hexane (10, 5, 2.5, 1.25, and 0.625% wt/wt), each tested on ten unsexed adult large grain borers per replicate, with four replicates per treatment. The mortality data of the insects at each level of dilution were collected, and the mean values were computed and subjected to Student-Newman-Keuls (SNK) t-test. Essential oils were also tested as antifeedants of the insect in dried maize. It was observed that within the 0.625% level of dilution, the oil extracts showed a significant difference ( $p < 0.05$ ) in the antifeedant test done using the different plant essential oils. Of the five plant oils, *P. marrubiooides* and *T. riparia* showed the highest insecticidal activity with the lowest mean of  $0.55 \pm 0.15$  and  $0.40 \pm 0.11$ , respectively, as compared to *O. lamiifolium* ( $2.4 \pm 0.11$ ), *O. suave* ( $2.55 \pm 0.11$ ), and *L. javanica* ( $2.5 \pm 0.15$ ) after a period of six months. The chemical constituents of the two most potent oils were analyzed by GC-MS. The main compounds in *P. marrubiooides* essential oil were  $\delta$ -2-carene (10.84%), *o*-cymene (8.72%), 1,8-cineole (7.89%), and camphor (5.65%). *T. riparia* essential oil was found to be rich in fenchone (24.74%),  $\beta$ -pinene (5.23%), *o*-cymene (5.16%), and 1,8-cineole (10.89%). These results show that these plant oils, and particularly *P. marrubiooides* and *T. riparia*, have the potential to protect dry stored grain products from damage by the larger grain borer. Consequently, the plant oils can be developed into post-harvest control agents of the larger grain borer in stored grain products.

### ARTICLE INFO

**Keywords:**  
Essential oils,  
Contact toxicity,  
Feeding deterrence,  
*Prostephanus truncatus*.

#### Article History:

Received 24 February 2025

Received in revised form 15 September 2025

Accepted 18 September 2025

Available online 21 October 2025

### 1. Introduction

The Larger Grain Borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) (Fig. 1) is a serious insect pest of maize (*Zea mays*), cassava (*Manihot esculenta*), wood, and other stored products (Nang'ayo et al., 2002; Quellhorst et al., 2021). *Prostephanus truncatus* is a major post-harvest pest in sub-Saharan

Africa, causing estimated maize and dried cassava losses of up to 30-40%, with economic losses ranging from USD 18.2 billion to USD 78.9 million annually between the years 1970 and 2020 (Diagne et al., 2021), depending on infestation levels and control strategies. The latest report on the occurrence of *P. truncatus* in Botswana follows similar reports in neighboring countries, Zambia,

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Editor: Francis Orata, Masinde Muliro University of Science and Technology, Kenya.

Citation: Adhiambo J., Ochieno D., & Omolo M. (2025). Feeding Deterrence and Insecticidal Activity of Selected Plant Essential Oils Against the Larger Grain Borer *Prostephanus truncatus* in Stored Maize Grains. Journal of Advances in Science, Engineering and Technology 2(1), 51 – 61.

Namibia, South Africa, and Zimbabwe in 1993, 1998, 1999, and 2005, respectively (Quellhorst et al., 2021). This invasive insect pest was introduced from its native Meso-American region into East Africa through Tanzania in the late 1970s (Dunstan & Magazini, 1981; Farrell & Schulten, 2002; Quellhorst et al., 2021). This devastating insect pest is a biosecurity threat that continues to spread around the world (Quellhorst et al., 2022), having already been recorded in 21 African countries among at least 36 reported territories globally (Quellhorst et al., 2021). Adult *Prostephanus truncatus* attacks maize, creating large and noticeable holes that ultimately 'skeletonize' the grains (Kumar, 2002; Quellhorst et al., 2021) (Fig. 2). Larval *Prostephanus truncatus* feed mainly on the floury endosperm and the germ tissues (Vowotor et al., 1998). *Prostephanus truncatus* is among stored product pests that include the square-necked grain beetle, *Cathartus quadricollis* (Silvanidae), and the maize weevil, *Sitophilus zeamais* (Curculionidae), that have also been reported to facilitate colonization of stored grains by mycotoxicogenic fungi such as *Aspergillus* spp. and *Fusarium* spp. that produce aflatoxins and fumonisins (Lamboni & Hell, 2009; Kheseli et al., 2011; Danso et al., 2018). The Larger Grain Borer *Prostephanus truncatus* can cause maize postharvest losses as high as 56.7% within six months of storage and hence is a serious threat to food security and nutrition (Bezabih et al., 2022).

Control of *Prostephanus truncatus* has been a challenge (Quellhorst et al., 2022), and various control options are being considered for Integrated Pest Management (IPM) of this damaging insect pest (Tanda et al., 2022). Biopesticides, especially those based on insecticidal plants (Okwute, 2012; Zoubiri & Baaliouamer, 2014; Riyaz et al., 2022), have been attracting a lot of interest in the management of *P. truncatus* and other stored products pests (Mantzoukas et al., 2020; Gariba et al., 2021; Tanda et al., 2022). Among the main reasons behind the growing interest in insecticidal plants is their affordability and safety to human and animal health, besides not posing adverse effects to non-target biodiversity and the environment (Ponsankar et al., 2016; Riyaz et al., 2022).

Various insecticidal plant species around the world are being investigated for their capacity to control *Prostephanus truncatus* (Bezabih et al., 2022). Such plant species include Horsewood or 'Maggot Killer' (*Clausena anisata*; Rutaceae), Silver Thistle (*Carlina acaulis*; Asteraceae), Lenten Rose (*Helleborus odorus*; Ranunculaceae), and *Equilabium glandulosum* (*Plectranthus glandulosus*; Lamiaceae), which has had nomenclatural disharmonies (Lukhoba et al., 2006; Nukenine et al., 2010; Rice et al., 2011; Kavallieratos et al., 2020; Mantzoukas et al., 2022). Besides, these are pesticidal plant species that have shown potential against mosquitoes (Culicidae) among other blood-feeding insects, as well as arachnid pests (Omolo et al., 2004; Omolo et al., 2022). These plants, traditionally used in pest control, such as those from the families Asteraceae, Lamiaceae, Solanaceae, and Verbenaceae, have demonstrated significant insecticidal properties.

These plants are often rich in essential oils containing diverse bioactive compounds with pesticidal potential (Omolo et al., 2004; Ngari et al., 2019; Omolo et al., 2022). Essential oils and powdered vegetative material from such plants contain various insecticidal and repellent chemical compounds that could be formulated into biopesticides.

The present study investigated the feeding deterrence and contact toxicity of essential oils from *Lippia javanica*, *Ocimum lamiifolium*, *Ocimum suave*, *Plectranthus marrubiooides*, and *Tetradenia riparia* against *Prostephanus. truncatus*. The results of the findings form a basis for the development of biopesticides for insect pests of stored food products and *P. truncatus* in particular.



Fig. 1. Adult *Prostephanus truncatus* (A) (Gueye et al., 2008)

## 2. Materials and Methods

### 2.1. Plant material

Fresh aerial plant parts that included leaves, stems, and flowers of *L. javanica*, *O. suave*, *O. lamiifolium*, *P. marrubiooides*, and *T. riparia* were collected early in the morning from mature, healthy plants using sterilized secateurs and clean gloves within Baringo and Nakuru counties in Rift Valley, Kenya. Stems of 10-15 cm in length were selectively harvested to ensure uniformity across species. The plant materials were kept under shade, and their oils were extracted within 24 hours of their collection.

### 2.2. Experimental insects

Experimental insects were derived from a colony of *P. truncatus* maintained at the Centre for African Medicinal and Nutritional Flora and Fauna (CAMNFF) at Masinde Muliro University of Science and Technology (MMUST). The insects were reared on maize seeds (Variety DK 8031, Kenya Seed Company Ltd, Kitale) in 1-liter glass jars with meshed covers and placed in darkness under prevailing laboratory conditions (25 ± 1°C; RH 70-75%).

### 2.3. Essential oil extraction

A total of 500 g of aerial parts from *Lippia javanica*, *Ocimum suave*, *Ocimum lamiifolium*, *Plectranthus marrubiooides*, and *Tetradenia riparia* were subjected to hydro-distillation for 6 hours using a Dean-Stark apparatus to minimize loss of thermolabile volatile compounds, following the method of Eisenbraun & Payne (1999). Plant material was immersed in water and heated to boiling, allowing volatile compounds (essential oils) to evaporate with the steam. The vapor was then condensed, and the immiscible oil layer separated from

the water. The extracted essential oils were dried over anhydrous sodium sulfate ( $\text{Na}_2\text{SO}_4$ ) to remove residual moisture. The essential oils were collected in 5 mL amber-colored glass vials to protect them from light degradation and stored at 4 °C for subsequent bioassays.



Fig. 2: Damaged maize grain from *P. truncatus* feeding (B) (Kumar, 2002).

#### 2.4. Fumigant and contact toxicity of plant leaves on *Prostephanus truncatus*

Dried maize grains (100 g) mixed with the test plant leaves (20 g) were placed in a transparent plastic jar (500 mL) with four sets of 20 pin holes on four upper sides for aeration. Unsexed adult *P. truncatus* ( $n=10$ ) were introduced into the jar in four replicates per treatment, including a control comprising maize grains without the leaves. After every 28 days, dead and surviving *P. truncatus* were counted for a period of 6 months.

#### 2.5. Feeding deterrence assay

Feeding deterrence activity was assessed in terms of reduced consumption of maize grains in the form of damage and the number of holes. Dried maize seeds ( $n=30$ ) were treated for 10 seconds with the oils dissolved in hexane at concentrations (wt/wt) of 10, 5, 2.5, 1.25, and 0.625 %, while the positive control maize received hexane alone, and the negative control was left without any treatment. Both treated and control maize grains were air dried at room temperature for 30 min to allow the solvent to evaporate and then placed in transparent plastic jars (500 mL). Adult *P. truncatus*

( $n=5$ ) were introduced into each jar before the maize grains were added, and the tests were done in four replicates arranged in a randomized block design (RBD) on a bench. Tests were conducted in four replicates across the treatment concentrations. Each replicate consisted of a separate test jar, and unsexed adult insects were used once per replicate without reusing between treatments. The insects were reared in a dark room under laboratory conditions ( $25 \pm 1^\circ\text{C}$ ; RH 70-75%). After 28 days, dead and live *P. truncatus* were counted. The total number of destroyed seeds and holes per seed was used to estimate consumption and hence damage to the maize.

#### 2.6. Analysis of the oil constituents

Analyses of the oils and identification of the chemical components were carried out using an Agilent Gas Chromatograph (GC) (Model 7890B, Country of origin?) coupled to a Quadropolar Mass Spectrometer (QMS) (Model 5977A, Agilent Technologies, US) and GC co-injection of the essential oils with authentic samples (Nangari et al., 2019). Analyses were performed on a capillary Gas Chromatograph (Hewlett Packard (HP) 5890 Series II, equipped with a split-less capillary injector system, 50m·0.2mm (I.d.) crossed-linked methyl silicone (0.33  $\mu\text{m}$  film thickness) capillary column, and flame ionization detector (FID) coupled to HP 3393A Series II integrator. The carrier gas was  $\text{N}_2$  at 0.7 mL  $\text{min}^{-1}$ . The temperature program consisted of an initial 50 °C (5 min) to 280 °C at 5 °C  $\text{min}^{-1}$  and held at this temperature for 10 min.

Gas Chromatography-Mass Spectrometry (GC-MS) analyses were carried out on an HP 8060 Series II Gas Chromatograph coupled to a VG Platform II Mass Spectrometer. The MS was operated in the EI mode at 70 eV and an emission current of 200  $\mu\text{A}$ . The temperature of the source was held at 180 °C and the multiplier voltage at 300 V. The pressure of the ion source and MS detector were held at  $9.4 \times 10^{-6}$  and  $1.4 \times 10^{-5}$  mbar, respectively. The MS had a scan cycle of 1.5s (scan duration of 1s and inter-scan delay of 0.5s). The mass and scan ranges were set at m/z 1-1400 and 38-650, respectively. The instrument was calibrated using hepta-cosafluorotributyl amine,  $[\text{CF}_3\text{-}(\text{CF}_2)_3]_3\text{N}$ , (Apollo Scientific Ltd, UK). The column used for GC-MS was the same as the one described for GC analysis, except for the film thickness (0.5  $\mu\text{m}$ ). The temperature programme involved an initial temperature of 50 °C (5 min), to 90 °C at 5 °C  $\text{min}^{-1}$ , to 200 °C at 2 °C  $\text{min}^{-1}$ , to 280 °C at 20 °C  $\text{min}^{-1}$ , and a hold at this temperature for 20 min. Identification of the components was made by comparison of mass spectra with published data (NIST, Wiley) and confirmed, where possible, by GC co-injections with authentic samples.

#### 2.7. Statistical analysis

Data on *P. truncatus* mortality in the fumigant and contact toxicity assay, as well as data on grain damage and number of holes in the feeding deterrence assay, were subjected to Shapiro wilk test for normality test before subjecting to analysis of variance (ANOVA) in Proc GLM and the means separated using Student-Newman-

Keuls (SNK) t-test in Statistical Analysis System (SAS versions 9.1) (SAS, 2004). Data on *P. truncatus* mortality at different concentrations of essential oils in the feeding deterrence assay were log-transformed and subjected to Probit regression analysis to determine lethal concentrations (LC<sub>75</sub> and LC<sub>95</sub>) using EPA Probit Analysis Program version 1.4 (US EPA, 1988).

### 3. Results and Discussion

Mortality of *P. truncatus* was significantly higher in maize grains with leaf material from all five tested

plant species compared to the controls by the end of the first month (Table 1), which confirms the pesticidal potential of *L. javanica*, *O. lamiifolia*, *O. suave*, *P. marrubioides*, and *T. riparia* leaves (Bekele et al., 1996; Omolo et al., 2004; Zoubiri & Baaliouamer, 2014; Kamanula et al., 2017; Ejeta et al., 2021). By the end of the fifth month, mortality of *P. truncatus* was highest in maize grains that were treated with *P. marrubioides* and *T. riparia* leaves, compared to those treated with *L. javanica*, *O. lamiifolium*, and *O. suave* (Table 1).

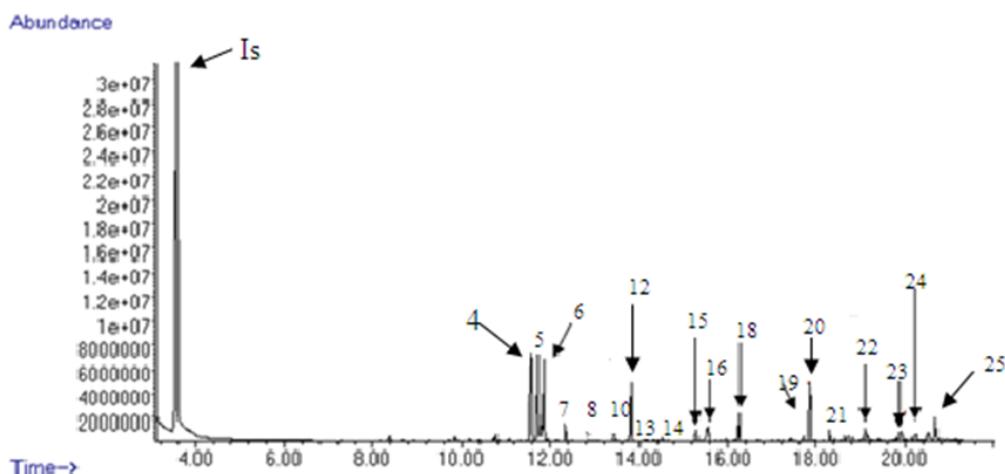
**Table 1:** Mortalities (Mean  $\pm$  SE) of *Prostephanus truncatus* treated with essential oils from different plant species over a five-month period.

Treatment	Mortalities				
	Month 1	Month 2	Month 3	Month 4	Month 5
Control	0.50 $\pm$ 0.28 b	0.50 $\pm$ 0.28 c	0.50 $\pm$ 0.29 c	0.75 $\pm$ 0.25 c	0.75 $\pm$ 0.25 c
<i>Lipia javanica</i>	1.75 $\pm$ 0.25 a	2.00 $\pm$ 0.08 b	2.00 $\pm$ 0.08 b	2.75 $\pm$ 0.25 b	3.00 $\pm$ 0.41 b
<i>Ocimum lamiifolia</i>	1.50 $\pm$ 0.64 a	1.50 $\pm$ 0.65 b	2.00 $\pm$ 0.41 b	2.75 $\pm$ 0.25 b	2.50 $\pm$ 0.29 b
<i>Ocimum suave</i>	1.25 $\pm$ 0.75 a	2.25 $\pm$ 0.63 b	2.50 $\pm$ 0.50 b	2.78 $\pm$ 0.48 b	2.75 $\pm$ 0.48 b
<i>Plectranthus marrubioides</i>	2.25 $\pm$ 0.25 a	3.50 $\pm$ 0.65 a	3.73 $\pm$ 0.48 a	3.75 $\pm$ 0.48 a	4.50 $\pm$ 0.64 a
<i>Tetradenia riparia</i>	1.75 $\pm$ 0.48 a	2.00 $\pm$ 0.41 b	3.00 $\pm$ 0.41 a	3.25 $\pm$ 0.25 a	3.75 $\pm$ 0.48 a

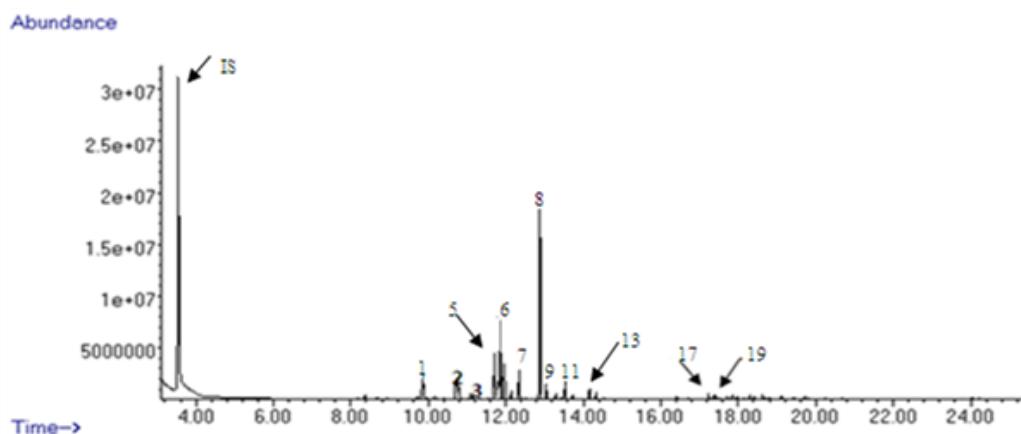
Means followed by the same letter within a column are not significantly different at P < 0.05 according to the student-Newman-Keuls (SNK) test.

Grain damage and the number of holes in the grains were lower in those treated with essential oils from all five plant species, especially after the first month to the sixth month (Table 2). Grain damage and the number of holes in the grains were lowest in maize grains that were treated with essential oils from *P. marrubioides* and *T. riparia*, compared to those treated with essential oils from *L. javanica*, *O. lamiifolium*, and *O. suave* (Table 2). Grain damage and the number of holes in the grains treated with hexane solvent were lower compared to the controls, with even further reduction in the two parameters when dissolved essential oils from any of the five plant species were present (Table 2). Essential oils from the five plant species exhibited varying efficacies, with *P. marrubioides* being the most effective as per the lowest lethal concentrations (LC<sub>75</sub> = 0.2; LC<sub>95</sub> = 1.8), followed by *T. riparia* (LC<sub>75</sub> = 5.6; LC<sub>95</sub> = 7.0), then *L. javanica* (LC<sub>75</sub> = 13.9; LC<sub>95</sub> = 21.0), *O. suave* (LC<sub>75</sub> = 55.1; LC<sub>95</sub> = 78.7) with *O. lamiifolium* having the highest lethal concentration and hence the weakest (LC<sub>75</sub> = 231.3; LC<sub>95</sub> = 324.7). Of the nineteen (19) and twelve (12) compounds identified in *Plectranthus marrubioides* and in *Tetradenia riparia*, respectively (Fig. 3 & 4: Table 3), six (6) compounds present in both plant species included *o*-Cymene C<sub>10</sub>H<sub>14</sub>, 1,8-Cineole C<sub>10</sub>H<sub>18</sub>O,  $\gamma$ -Terpinene C<sub>10</sub>H<sub>16</sub>, Fenchone C<sub>10</sub>H<sub>16</sub>O, Borneol C<sub>10</sub>H<sub>18</sub>O, and (E)  $\beta$ -Caryophyllene C<sub>15</sub>H<sub>24</sub>. Some of these compounds have been detected in the genera of the two plant species (Abdel-Mogib et al., 2002; Lukhoba et al., 2006; Blythe et al., 2020), and may include those especially in *Plectranthus* spp, whose chemistry has not been well known (Abdel-Mogib et al., 2002; Lukhoba et al., 2006). The compound *o*-cymene exhibits insecticidal activity through strong fumigant and contact toxicity (Feng et al., 2021), which has been demonstrated against *Tribolium castaneum* and *Liposcelis bostrychophila* (Feng et al., 2021).

However, *o*-cymene has also been found to be attractive to honey bees (Fernandes et al., 2019; Dekebo et al., 2022). The compound 1,8-Cineole exhibits fumigant toxicity as observed against *Musca domestica* (Rossi & Palacios, 2015). Fenchone is part of bioactive chemical compounds in essential oils of *Lavandula dentata* (Lamiaceae), which have been found to exhibit insecticidal activities towards *Sitophilus zeamais*, *Tribolium castaneum*, and *Epicauta atomaria* (Wagner et al., 2021). Insecticidal mechanisms of action 1,8-Cineole and Fenchone include inhibition of acetylcholinesterase (AChE) and cytochrome P450 system, blockage of  $\gamma$ -aminobutyric acid (GABA)-gated chloride channels, and agonist of octopamine receptors (Rossi and Palacios, 2015; Sengül Demirak & Canpolat, 2022). The  $\gamma$ -terpinene compound exhibits larvicidal and ovicidal activities against the cotton bollworm (*Helicoverpa armigera*) (Gong & Ren, 2020). Borneol has been found to inflict mortality on red imported fire ant (*Solenopsis Invicta*) through fumigant toxicity (Zhang et al., 2014). (E)- $\beta$ -Caryophyllene and Caryophyllene oxide exhibit insecticidal, antifeedant, and growth inhibition activities (Langenheim, 1994; Huang et al., 2012; Liu et al., 2012), while acting as a nerve poison to pests through anticholinesterase and via sodium channel modulators, among other mechanisms of action (Liu et al., 2012; Paventi et al., 2020; Sengül Demirak & Canpolat, 2022). Information has been scarce on possible insecticidal mechanisms of Sylvesterene,  $\delta$ -Cadinene, and  $\gamma$ -Murolene; as well as whether the remaining three compounds, 7-oxabicyclo [4.1.0] heptan-2-one, 3-methyl, Allo aromadendrene epoxide, and 4-hydroxy,1H indole-3-carboxylic acid detected in *P. marrubioides*, have any insecticidal potential. The other compounds and their mode of action are summarized (Table 4).



**Fig. 3:** Total ion chromatogram (TIC) obtained by coupled GC- MS analysis of vacuum distillates of *Plectranthus marrubioiodes*. Peak numbers correlate to compounds listed in Table 3



**Fig. 4:** Total ion chromatogram (TIC) obtained by coupled GC- MS analysis of vacuum distillates of *T. riparia*. Peak numbers correlate to compounds listed in Table 3.

*Tetradenia riparia* expressed six other chemical compounds that included  $\alpha$ -Pinene  $C_{10}H_{16}$ ,  $\beta$ -Pinene  $C_{10}H_{16}$ , 5-methyl, 3-hepten-2-one  $C_8H_{14}O$ , E-Ocimene  $C_{10}H_{16}$ , Allo Ocimene  $C_{10}H_{16}$ , and  $\beta$ -Bourbonene  $C_{15}H_{24}$ . The insecticidal effects of  $\alpha$ -Pinene and  $\beta$ -Pinene have been associated with mechanisms of action that include inhibition of AChE and cytochrome P450 system, blockage of GABA-gated chloride channels, and agonist of octopamine receptors (Sengül Demirak & Canpolat, 2022).  $\alpha$ -Pinene exhibits a repellent effect to honey bees (Fernandes et al., 2019; Dekebo et al., 2022).  $\beta$ -Bourbonene and (E)- $\beta$ -ocimene are volatile organic compounds (VOCs) used in indirect plant defenses through attraction of natural enemies towards insect-attacked host plants (Takabayashi et al., 1991; Klimm et al., 2020), while (E)- $\beta$ -ocimene also attracts pollinators towards floral parts (Knudsen et al., 2004; Tan & Nishida, 2012; Farré-Armengol et al., 2017). E- $\beta$ -ocimene also partially inhibits ovary development in the worker caste of the honey bees *Apis mellifera* (Maisonnasse et al., 2010). Both  $\beta$ -ocimene and allo-ocimene are volatile pheromones that are the primary signals of *A. mellifera* larvae used in begging for care from the nurses through odorant binding proteins (OBPs)/chemosensory proteins (CSPs) (Maisonnasse et al., 2010; Wu et al., 2019). There

is a scarcity of information on possible insecticidal activities of 5-methyl-3-hepten-2-one detected in *T. riparia*.

The general trends in the results of this study indicated that *P. marrubioiodes* and *T. riparia* inflicted the highest *P. truncatus* mortality, with the least grain damage among the five plant species. Although without statistically significant differences, numerical trends throughout the 6 months indicate that pesticidal compounds of *P. marrubioiodes* inflicted higher mortalities (Table 1), while those of *T. riparia* had the best effect in reducing grain damage (Table 2). These differences were partly confirmed in the efficacy tests, whereby essential oils of *P. marrubioiodes* had the lowest lethal concentration and hence the most toxic ( $LC_{75} = 0.2$ ;  $LC_{95} = 1.8$ ) compared to *T. riparia* ( $LC_{75} = 5.6$ ;  $LC_{95} = 7.0$ ).

The six chemical compounds common in both *P. marrubioiodes* and *T. riparia* included  $\alpha$ -Cymene, 1,8-Cineole,  $\gamma$ -Terpinene, Fenchone, Borneol, and (E)  $\beta$ -Caryophyllene, and accounted for 25% and 44.8% total concentration in *P. marrubioiodes* and *T. riparia*, respectively. Trends in the concentrations of these six

**Table 2:** Mean  $\pm$  SE of seed damage and number of holes by *Callosobruchus maculatus* over six months

Treatment	Month 1		Month 2		Month 3		Month 4		Month 5		Month 6	
	Damage	Holes	Damage	Holes	Damage	Holes	Damage	Holes	Damage	Holes	Damage	Holes
Control	4.0 $\pm$ 0.4 a	31.3 $\pm$ 6.0 a	6.3 $\pm$ 0.5 a	60.0 $\pm$ 1.8 a	9.0 $\pm$ 0.4 a	73.0 $\pm$ 1.4 a	10.8 $\pm$ 0.5 a	79.3 $\pm$ 3.3 a	14.5 $\pm$ 1.6 a	86.3 $\pm$ 3.4 a	16.3 $\pm$ 1.1 a	91.8 $\pm$ 4.5 a
Hexane	1.3 $\pm$ 0.3 b	3.8 $\pm$ 0.9 b	3.0 $\pm$ 0.4 b	27.0 $\pm$ 4.8 b	3.3 $\pm$ 0.3 b	40.0 $\pm$ 4.4 b	4.3 $\pm$ 0.8 b	46.8 $\pm$ 4.6 b	5.5 $\pm$ 0.7 b	53.5 $\pm$ 5.0 b	7.8 $\pm$ 0.8 b	61.3 $\pm$ 3.8 b
<i>L. javanica</i> <sup>1</sup>	0.8 $\pm$ 0.2 b	0.9 $\pm$ 0.2 c	1.4 $\pm$ 0.2 d	1.5 $\pm$ 0.2 d	1.6 $\pm$ 0.1 d	2.6 $\pm$ 0.3 c	1.8 $\pm$ 0.1 c	3.0 $\pm$ 0.3 c	2.1 $\pm$ 0.2 c	3.6 $\pm$ 0.4 c	2.5 $\pm$ 0.2 c	4.1 $\pm$ 0.4 c
<i>O. lamiifolia</i> <sup>2</sup>	1.2 $\pm$ 0.1 b	1.2 $\pm$ 0.1 c	1.8 $\pm$ 0.1 c	2.1 $\pm$ 0.1 c	1.9 $\pm$ 0.1 c	2.3 $\pm$ 0.1 c	2.0 $\pm$ 0.1 c	2.6 $\pm$ 0.1 c	2.3 $\pm$ 0.1 c	2.9 $\pm$ 0.2 d	2.4 $\pm$ 0.1 c	3.1 $\pm$ 0.1 d
<i>O. suave</i> <sup>3</sup>	1.4 $\pm$ 0.1 b	1.5 $\pm$ 0.2 c	1.6 $\pm$ 0.2 c	2.1 $\pm$ 0.2 c	1.9 $\pm$ 0.1 c	2.6 $\pm$ 0.2 c	2.0 $\pm$ 0.1 c	2.9 $\pm$ 0.2 c	2.4 $\pm$ 0.1 c	3.2 $\pm$ 0.2 c	2.6 $\pm$ 0.1 c	3.6 $\pm$ 0.2 c
<i>P. marrubioiodes</i> <sup>4</sup>	0.1 $\pm$ 0.1 c	0.1 $\pm$ 0.1 d	0.3 $\pm$ 0.1 e	0.4 $\pm$ 0.1 e	0.5 $\pm$ 0.2 e	0.5 $\pm$ 0.2 d	0.5 $\pm$ 0.2 d	0.6 $\pm$ 0.2 d	0.6 $\pm$ 0.2 d	0.6 $\pm$ 0.2 e	0.6 $\pm$ 0.2 d	0.7 $\pm$ 0.2 e
<i>T. riparia</i> <sup>5</sup>	0.0 $\pm$ 0.0 c	0.0 $\pm$ 0.0 e	0.2 $\pm$ 0.1 e	0.2 $\pm$ 0.1 e	0.3 $\pm$ 0.1 e	0.4 $\pm$ 0.1 d	0.4 $\pm$ 0.1 d	0.5 $\pm$ 0.1 d	0.4 $\pm$ 0.1 d	0.5 $\pm$ 0.1 e	0.4 $\pm$ 0.1 d	0.5 $\pm$ 0.2 e

Values followed by different letters within the same column are significantly different ( $p < 0.05$ ). Damage refers to the number of seeds visibly damaged by bruchid activity, while holes indicate perforations on seed surfaces. Lethal concentrations (LC<sub>75</sub>; LC<sub>90</sub>) for <sup>1</sup>*L. javanica* (13.9; 21.0), <sup>2</sup>*O. lamiifolia* (231.3; 324.7), <sup>3</sup>*O. suave* (55.1; 78.7), <sup>4</sup>*P. marrubioiodes* (0.2; 1.8), and <sup>5</sup>*T. riparia* (5.6; 7.0).

**Table 3:** The chemical compositions of essential oils of *Plectranthus marrubioiodes* and *Tetradenia riparia* plants

<i>Plectranthus marrubioiodes</i>				<i>Tetradenia riparia</i>			
Peak	Retention time	% Area	Peak identity	Retention time	% Area	Peak identity	
1	11.58	10.84	$\delta$ -2- Carene	9.87	2.92	$\alpha$ -Pinene	
2	11.73	8.72	$\alpha$ -Cymene*	10.77	5.23	$\beta$ - Pinene	
3	11.84	7.89	1,8- Cineole*	11.17	0.57	5-methyl, 3-hepten-2-one	
4	12.34	1.47	$\gamma$ -Terpinene*	11.73	5.16	$\alpha$ -Cymene*	
5	12.9	0.81	Fenchone*	11.84	10.89	1,8-Cineole*	
6	13.43	0.69	Cyclooctanone	12.34	1.89	$\gamma$ - Terpinene*	
7	13.84	5.65	Camphor	12.9	24.74	Fenchone*	
8	14.17	0.32	Borneol, heptafluorobutyrate*	13.05	1.55	E-Ocimene	
9	14.33	0.61	Terpinen-4-ol	13.52	1.7	Allo Ocimene	
10	15.29	1.02	iso-Sylvesterene	14.17	1.51	Borneol*	
11	15.56	2.25	7-oxabicyclo [4.1.0] heptan-2-one,3methyl	17.4	1.01	$\beta$ -Bourbonene	
12	17.44	0.47	Germacrene A	17.71	0.63	(E)-Caryophyllene*	
13	17.87	5.79	(E)-Caryophyllene*	-	-	-	
14	18.29	0.89	$\alpha$ -Humulene	-	-	-	
15	19.12	1.26	$\delta$ - Cadinene	-	-	-	
16	19.82	1.45	Allo aromadendrene epoxide	-	-	-	
17	20.27	1.34	4 Hydroxy, 1H indole -3-carboxylic acid	-	-	-	
18	20.69	2.88	$\alpha$ -Cadinol	-	-	-	
19	21.14	0.54	$\gamma$ -Murolene	-	-	-	

\*Chemical compound exists in both plant species

chemical compounds indicate that, apart from o-Cymene, which was in higher concentration in *P. marrubiooides*, the other five compounds, and especially Fenchone, were in higher concentrations in *T. riparia*. Furthermore, with a smaller number of compounds detected in *T. riparia*, those twelve (12) compounds accounted for 57.8% of the total concentration, while the nineteen (19) compounds detected in *P. marrubiooides* accounted for a lower concentration of 54.9%. The diversity and concentration of chemical compounds in the essential oils of *Plectranthus marrubiooides* may suggest the potential for synergistic interactions contributing to the observed insecticidal efficacy. However, such synergism was not directly assessed in the present study and remains a subject for

future investigation, when compared to *T. riparia*, whose efficacy may have been more reliant on the high concentration of Fenchone (24.74%). However, this assumption does not exclude the possible involvement of other insecticidal chemical compounds in the two plant species, including alkaloids (Rattray & Van Wyk, 2021), which may not have been detected in the GC-MS analyses applied in the current study. Essential oils of *P. marrubiooides* and *T. riparia* show insecticidal and feeding deterrent effects against *P. truncatus*, supporting their potential as biopesticides. However, formulation and delivery remain unknown. Future studies should assess formulation stability, safety, and field applicability.

**Table 4:** *Plectranthus marrubiooides* chemical compounds and their mode of action.

Compound name	Mode of Action / Effect	References
Camphor (C <sub>10</sub> H <sub>16</sub> O)	Contact & fumigant toxicity to <i>S.granarius</i> , <i>S.zeamais</i> , <i>T.castaneum</i> , <i>P.truncatus</i> AChE inhibition	(Obeng-Ofori et al., 1998)
δ 2-Carene (C <sub>10</sub> H <sub>16</sub> )	Fumigant toxicity to <i>C. maculatus</i> , <i>S. oryzae</i> , <i>T. castaneum</i> ; AChE inhibition	(Hashemi & Safavi, 2012)
α-Cadinol (C <sub>15</sub> H <sub>26</sub> O)	AChE inhibition	(Rants'o et al., 2022)
Terpinen-4-ol (C <sub>10</sub> H <sub>18</sub> O)	Toxic to Oriental armyworm via P450, GSTs, PPO inhibition	(Zhang, 2008)
iso-Sylvesterene (C <sub>10</sub> H <sub>16</sub> )	Contact toxicity; causes mortality and developmental disruption in <i>D. peruvianus</i> , <i>An.gambiae</i> , <i>Cx. quinquefasciatus</i>	(Pacheco et al., 2020)
Germacrene A (C <sub>15</sub> H <sub>24</sub> )	Indirect defense via attraction of predators; aphid alarm pheromone precursor	(Huber et al., 2016)
Cyclooctanone (C <sub>8</sub> H <sub>14</sub> O)	Alarm pheromone in ants ( <i>Pogonomyrmex badius</i> )	(Blum, 1969; Blum et al., 1971)
α-Humulene (C <sub>15</sub> H <sub>24</sub> )	Contact toxicity; inhibits respiratory chain complexes II-IV	(Kim & Lee, 2014; Paventi et al., 2020; Plata-Rueda et al., 2018)
δ-Cadinene (C <sub>15</sub> H <sub>24</sub> )	Insecticidal activity against <i>Bruchus dentipes</i> , <i>An. stephensi</i> , <i>Ae. aegypti</i> , <i>Cx. quinquefasciatus</i>	(Govindarajan & Benelli, 2016; Tozlu et al., 2011; Vaglica et al., 2022)
γ-Muurolene (C <sub>15</sub> H <sub>24</sub> )	Toxicity to <i>Bruchus dentipes</i>	(Vaglica et al., 2022)

## Acknowledgements

This work was supported by the University Research Fund of MMUST.

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