

# TOTAL PHENOLIC AND FLAVONOID CONTENT IN THE ESSENTIAL OILS OF SELECTED PLANTS AND ITS INSECTICIDE ACTIVITY FOR SUSTAINABLE CONTROL OF RED PALM WEEVIL (*Rhynchophorus ferrugineus*)

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**Abstract:** Essential oils offer a promising alternative to chemical pesticides, which pose environmental and health risks. However, comprehensive field studies evaluating their long-term effectiveness and practical application in diverse agricultural settings are lacking. This study investigates the insecticidal efficacy of essential oils from four plants: *Cymbopogon citratus* (lemongrass), *Melaleuca cajuputi* (gelam), *Vitex rotundifolia* (beach vitex), and *Pandanus amaryllifolius* (pandan) to control the red palm weevil (*Rhynchophorus ferrugineus*, RPW). The oils were assessed for their antifeedant and repellent properties to contribute to sustainable pest management strategies. Gelam oil had the highest levels of phenolics and flavonoids while pandan oil had the lowest. Prickmark analysis revealed that lemongrass oil had the greatest deterrent effect against RPW feeding, followed by gelam and beach vitex, with pandan oil showing no antifeedant activity. A Y-tube olfactometer evaluated the repellent effectiveness of the oils. Lemongrass and beach vitex had slightly attractive characteristics while gelam and pandan oils effectively repelled RPWs. Male and female RPWs responded differently: Males were more attracted to lemongrass and beach vitex while females were more repulsed by gelam and pandan. These findings suggest that essential oils' insecticidal properties could help develop gender-specific and sustainable agricultural pest management plans.

Keywords: Botanical insecticide, *Rhynchophorus ferrugineus*, phytochemical, feeding deterrent, repellent.

## Introduction

Half of the one million bug species are plant-eating insects (Wu & Baldwin, 2010). Millions of Malaysian Ringgit are wasted annually in agriculture due to these pests; thus, when it comes to natural and planted crops, this can have a significant ecological and economic impact. One such pest is the red palm weevil (RPW), scientific name *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae). It is a significant invasive tissue borer with a vast host range restricted to palm trees, infesting roughly 17 palm species globally, including oil palm and coconut. According to Wahizatul *et al.* (2013), the importation of illegal date palm trees in 2007 is what led to the invasion of RPW, particularly in Malaysia. In Malaysia, tracking of the RPW

population began in Terengganu state in 2011 and spread quickly to the rest of Peninsular Malaysia within a decade (Yan *et al.*, 2021). During the initial stages of an infestation, it can be extremely difficult to locate the RPW. The most formidable threat is an unseen adversary that inflicts damage from within, leading to the demise of the palm tree within six to eight months. Once the palm is affected, its survival becomes unattainable. By the time the presence of the weevil is identified, it is often too late to intervene and save the palm.

The current Integrated Pest Management (IPM) for RPW in Malaysia heavily relies on chemicals (Malaysian Department of

Agriculture, 2017). For example, chemical insecticides such as Spirotetramat, Fipronil, Chlorpyrifos, and Methidathion can be applied to RPW-infested palms using the trunk injection method (Shar *et al.*, 2012) or by spraying cypermethrin on their canopy for some shorter palm plants (Wahizatul *et al.*, 2017). While using chemical or synthetic insecticides can cause an almost complete removal of the pests from the infested area, they negatively influence the natural environment and human health (Chowański *et al.*, 2014). These insecticides can deteriorate ecosystems and human health due to their high toxicity and persistence properties. Thus, the overuse of synthetic insecticides has been a matter of concern for both scientists and the public in recent years. To reduce negative impacts on the environment and human health, natural products with insecticidal characteristics are an excellent alternative to synthetic insecticides. Plant volatiles are essential in communication between host plants and herbivorous insects (Zu *et al.*, 2023). Plants also have built-in defence mechanisms against herbivores and undesirable pests in the form of secondary metabolites known as Essential Oils (EOs). Over the years, EOs have been popular as insecticides since they are safe for humans and the environment, they have low persistence under field conditions, tend to be relatively nontoxic to non-target subjects, leave little or no residues on food are durable in use as the target pests are less likely to evolve resistance, and they are inexpensive (Isman, 2020).

EOs have been proven by many researchers to exhibit insecticidal activities such as repellency, contact toxicity, ovicidal, larvicidal, and antifeedant against important pests. EOs can easily enter an insect's respiratory system due to their highly volatile nature. EOs can also interact with many different olfactory receptor systems of insects, eliciting various physiological responses (Luker *et al.*, 2023). To name a few, cinnamon's EO exhibits repellent activity against the rice weevil (Shi *et al.*, 2022) while basil oil showed the strongest contact toxicity effect on the third instar larvae of the tobacco cutworm compared to the other 27 EOs

studied by Kim *et al.* (2021). On the other hand, EO from plants of the Lamiaceae family showed excellent larvicidal and adulticidal activities against the West Nile virus vector, *Culex pipiens* (El-Kasim Bosly, 2022). In a study by Valcárcel *et al.* (2021) on 14 aromatic plants' EOs against ticks, they identified that the EOs can be grouped into two based on their antifeedant effectiveness: Strong and moderate. They also conclude that the bioactive compounds within the EOs can exert the effect individually or synergistically. There were also studies done on the impact of botanical EOs on RPW. A survey by Mady *et al.* (2021) showed that 10% sweet marjoram EO can kill 50% of the population of RPW larvae, pupa, and adults within 96 hours, 48 hours, and 12 hours, respectively. Aziza and Mona (2016) reported that EO from *Juniperus communis* possesses contact and fumigant toxicity in all stages of RPW especially the eggs and larvae while inducing some biochemical changes in adults when used as contact poison. In addition, Ali *et al.* (2019) tested five different plant EOs, lemon, orange, basil, castor, and eucalyptus and concluded that they could control RPW in the egg and larva stage. Alternatively, *Syzygium aromaticum* and *Cinnamomum eucalyptus* oil revealed an oviposition deterrent effect against RPW (Saad, 2017). The antifeedant effect of eight EO derivatives was also evaluated against RPW larval stages by Yan *et al.* (2021).

The efficiency of the plant EOs as natural insecticides can be credited to their phytochemical composition, particularly the presence of high levels of phenolics and flavonoids. Benelli *et al.* (2018) reported that EOs obtained from the industrial hemp, *Cannabis sativa* dominated by monoterpenes and sesquiterpenes showed strong toxic effects against the housefly, *Musca domestica*. The presence of high levels of phenolic compounds in the Arabic gum tree, *Acacia nilotica* has also been implicated in the toxicity activity of this plant against tobacco cutworms (Gautam *et al.*, 2021). In addition, fruit extract of the cow's udder, *Solanum mammosum* which contains various phytochemicals including phenolics and flavonoids was found to be more effective in the

suppression of fruit fly's pupae eclosion than a commercial insecticide (Tran *et al.*, 2022).

In our study, RPWs were treated with EOs from four plants, namely *Cymbopogon citratus* (lemongrass), *Melaleuca cajuputi* (gelam), *Vitex rotundifolia* (beach vitex), and *Pandanus amaryllifolius* (pandan) to determine the potential of these EOs as antifeedant and repellency agents for sustainable RPW control. While Malaysians widely use lemongrass and pandan as insecticides, the use and study of the insecticidal characteristics of beach vitex and gelam are few. In addition, since EOs represent a complex and unique mixture of compounds specific to each plant and extraction procedure, it opens up vast opportunities for researchers to study their insecticidal activities and develop the best formulation for bioinsecticide against the targeted pest.

## Materials and Methods

### *Collection and Rearing of Red Palm Weevils (RPWs)*

The pheromone mass trapping of RPWs was done according to the method by Yan *et al.* (2021) with slight modifications. Pheromone traps were installed around infested areas in Kuala Nerus, Terengganu (5°22'17.6" N 103°04'52.5" E). Sampling sessions were conducted for three months, from June to November 2021. A designated 5 L polypropylene bucket with four holes on its upper part and a cover lid with hanging rope to hang the pheromone lure (Ferrolure P028+, ChemTica Int., Costa Rica) was used. Pineapple slices and water were placed into bucket traps to synergise the trapping effectiveness. Food baits and water levels were replenished each week. At the same time, trapped RPWs were collected and transferred to the Ecology Lab, Universiti Malaysia Terengganu (UMT) for the rearing process.

The collected adult RPWs were reared under *in vitro* conditions using a ventilated plastic container and sugarcane slices were provided as a food source. A seven-day acclimatisation

process at room temperature was taken, which allows adult RPWs to be accustomed to a new food source and environment before conducting a bioassay. Each RPW was examined for sex, physical condition, and behaviour. Gender can be determined by which male RPW has a brush rostrum and which female RPW has a slender rostrum. To get a reliable and accurate reading, RPWs with broken limbs (such as legs, antennae, or rostrum) or that were weak (moving or dying slowly) were not included in the bioassay. Selected test subjects were individually quarantined and starved for 24 hours. Each treatment was replicated three times, consisting of 10 adult RPWs with five males and five females.

### *Extraction of Essential Oils (EOs)*

Four plants selected in this study are *C. citratus* (lemongrass), *M. cajuputi* (gelam), *V. rotundifolia* (beach vitex), and *P. amaryllifolius* (pandan) while all these plants were obtainable in Kuala Nerus, Terengganu, Malaysia. Lemongrass and pandan were purchased from local markets, Suraya Grocery (5°23'45.3" N 103°05'38.3" E), and Pasar Nelayan (5°26'06.2" N 103°03'52.3" E), respectively. Wild gelam leaves and beach vitex leaves were collected from the *Melaleuca* forest in Bari Besar, Permaisuri, Terengganu, Malaysia (5°33'14.6" N 102°52'15.8" E) and the beach side of Tok Jembal Beach, Terengganu, Malaysia (5°24'19.0" N 103°05'52.1" E), respectively.

To extract the EOs from the plant samples, they were first washed and cut into smaller pieces to remove unnecessary objects and parts. Extraction of EOs was done using a hydro distillation process with hexane as the solvent. Distilled water was first filled to cover plant samples in the heating mantle. Liquid from the distillate was further processed with hexane in a separatory funnel, where separation of water and oil was formed. All the above procedures were repeated until sufficient EOs were obtained for bioassays. The collected EOs were stored in a glass vial and kept inside the refrigerator before analyses.

### ***Quantification of Total Phenolic and Flavonoid Contents of EO***

Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) for all four extracted plant EOs were determined using the Varian UV-Vis Spectrophotometer CARY 50 CONC, aided by the software Cary WinUV Concentration Application Version 5.0.0.999 (Agilent Technologies Inc.). All EO test samples and standards (gallic acid and quercetin) were performed in triplicate.

TPC was estimated using Folin-Ciocalteu's method according to Kamboj *et al.* (2015) with some modifications. One mL of the EO test sample was mixed into a test tube containing one mL of 10% Folin-Ciocalteu reagent. Two mL of 7.5% sodium carbonate were added to the test tube and shaken gently. The mixture was then incubated in a dark place for 90 minutes at room temperature until an intense blue colour developed. Then, the absorbance of the sample was measured at 750 nm. The spectrophotometric blank unit was performed using a reagent blank with solvent. Gallic acid (i.e., 0.001, 0.01, 0.02, 0.04, and 0.06 mg/mL) was used as standard. TPC was expressed as mg of Gallic Acid Equivalent (GAE) per gram of plant.

TFC was estimated using an aluminium chloride colourimetric assay (Dowd, 1959) with some modifications. Two mL of EO test sample was mixed with two mL of 2% aluminium chloride. Then, the test tube was shaken gently. The mixture was stored in a dark place for 60 minutes at room temperature until a yellow colour was developed. The absorbance was then measured at 430 nm. The spectrophotometer blank unit was performed using a reagent blank with solvent. Quercetin (i.e., 0.001, 0.01, 0.02, 0.04, and 0.06 mg/mL) was used as standard. TFC was expressed as mg of Quercetin Equivalent (QE) per gram of plant.

### ***Antifeedant Bioassay of Essential Oils against Red Palm Weevil***

For the antifeedant bioassay, all four extracted plant EOs were first diluted into concentrations of 5% and 10% solution with distilled water

and 2% Triton X-100 as an emulsifier. An aqueous solution with the same solvent and emulsifier was the positive control. Antifeedant bioassay for plant EOs against adult RPW was evaluated using the prick-marks counting method. The fresh sugarcane stem was split into two equal longitudinal halves, each with an exposed surface area of about 20.15 cm<sup>2</sup>, with a rectangular dimension of 8.5 cm x 2 cm and approximately 1.57 cm<sup>2</sup> for each semicircle side. Each sugarcane slice was treated with (approximately 0.8 mL) EO or control solution by dropper on its exposed surface. The treated sugarcane slice was then transferred into a ventilated plastic container (10 cm diameter x 5 cm height) for evaporation. A pair of adult RPW of different sexes were moved into the container with treated sugarcane slices. The container was then covered tightly using a transparent lid. Observations on feeding were taken for five days. Prick marks on the sugarcane were counted and recorded every 24 hours, and RPW condition was observed. The counting was aided by a square window of 2 x 2 cm<sup>2</sup>.

### ***Repellent Bioassay of Essential Oils against Red Palm Weevil***

A repellent bioassay of the EOs was carried out using a Y-tube olfactometer (Figure 1) according to the fabrication by Haris-Hussain *et al.* (2020). Chambers for EO samples and control were designated with a 3 mm transparent Perspex cube in dimensions of 20 x 20 x 20 cm<sup>3</sup> and the top base was an openable lid. An approximate 3 cm round hole was drilled in the middle of one lateral face of the cube. For the two chambers used to place treatment or control, three 15 cm length rectangular holes were designed (oppositely facing the round hole opening side) to allow a single airflow direction from small axial flow fans (wind speed: Approximately 6 ms<sup>-1</sup>). Rubber rims were used at the end of both arms of the Y-tube to connect the chambers.

The study tested four treatments: Lemongrass, gelam, beach vitex, and pandan

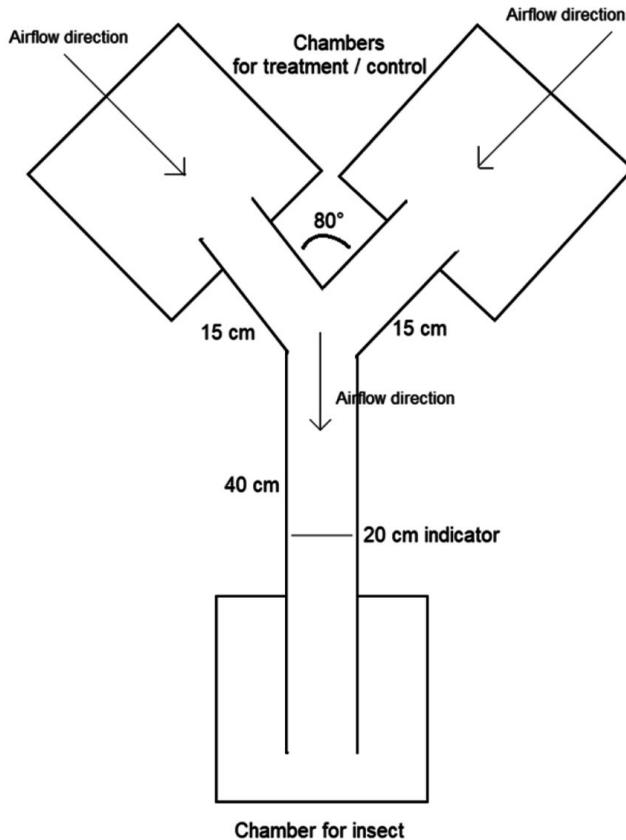


Figure 1: The Y-tube olfactometer for repellent bioassay

EO at a concentration of 10%. The positive control used contains the solvent with 2% Triton X-100. 300 g of sugarcanes were dipped with EO treatments or control (~7 mL) and then placed into the treatment chambers for the evaporation process for about one minute. 10 mL of EO solution or control in an uncovered glass vial was put into each chamber according to the same treatment the sugarcane had previously dipped.

Adult RPWs were acclimated and starved for one day before conducting the bioassay. One adult RPW was placed in the terminal end of the olfactometer. Time was counted once the RPW passed through the indicator line plotted in the terminal tube (Figure 1). Bioassay would be stopped if the RPW entered one of the branch arms and stayed for 1 minute while motionless. RPW for 15 minutes was recorded as a “no-choice” category. Data was not recorded if the

RPW did not move or pass through the indicator line for 15 minutes. One hundred adult RPWs (50 males and 50 females) were used for each treatment and the tested subjects were not used repeatedly. The tube or chambers were rotated/side-switched frequently during the bioassay to obtain accurate data. After each assay, all chambers and the tube were washed and rinsed with alcohol before conducting the next treatment.

The average Intensity of Reaction (IR) was then calculated according to this formula:  $\% IR = [(T - C)/(T + C)] * 100$ , where T is the number of RPW in the chamber containing the EOs and C is the number of RPW observed in the chamber containing the control (Sharaby & Al-Dosary, 2014). Percentage of IR ranging between 1% and 10% indicates that the oils are weakly attractive, 11% and 20% are attractive,

and greater than 40% are highly attractive. If less than 1% of the oils are considered repellent (Sharaby & Al-Dosary, 2014).

**Statistical Analyses**

Data analyses were done using the statistical program IBM SPSS Statistics version 20. TPC and TFC of each plant EO and prick-marks counting data were analysed by mean values from three replications in each treatment. Data was collected only if the RPW had survived more than two days. The comparison of TPC, TFC, and control to each treatment in different concentrations and each day was calculated through one-way ANOVA, followed by Tukey’s HSD post hoc test with a significant difference level at  $\alpha = 0.05$ .

**Results and Discussion**

Some plants possessed compounds that were repulsive to insects and had an offensive smell. For example, EOs are highly concentrated aromatic compounds extracted from plants. They are volatile and are often referred to as the “essence” of the plant from which they are derived. These substances play a significant part in plant defence against many pest species (Pavela & Benelli, 2016). EOs are made up of different chemical combinations. The phenolic and flavonoid compounds are important because they are made as secondary metabolites when a defence mechanism against phytophagous insects is activated. The TPC and TFC of the four plant EOs are listed in Table 1. TPC and TFC concentrations in gelam are substantially greater than those in the other three EOs

( $p < 0.05$ ). Lemongrass and beach vitex came in second and third, respectively while pandan displayed the lowest value.

Evolutionary processes may have an impact on plant phenolic and flavonoid concentrations. As a part of their defence systems against viruses, herbivores, or environmental stressors, plants have evolved to create these substances (Kumar et al., 2020; Chowdhary et al., 2022). The production of phenolics and flavonoids in plants can be influenced by environmental factors such as soil composition, temperature, light intensity, and the availability of nutrients and water (Yang et al., 2018). Thus, different plant species that belong to the same genus may adopt special and distinctive compositions in various circumstances or locations (Ali & Neda, 2011). For example, in this study, TPC from gelam was found to be lower than in other studies from Malaysia, which were conducted in the states of Kedah (37 0.05 GAE/mg extract dry weight) and Kelantan (16.53 0.00 mg/GAE/g) (Noor et al., 2021). In comparison to the findings of Mirghani et al. (2012), TPC and TFC in lemongrass EO were marginally lower. Pandan EOs from Kedah, Selangor, and Johor states in Malaysia were also determined; however, there was no report for TPC and TFC values in the study (Zakaria et al., 2020).

Among the TPC and TFCs, carvacrol, linalool, alpha-pinene, menthol, cinnamaldehyde, eugenol, 1-8 cineole, geraniol, and limonene are some of the components of EOs that have shown insecticidal activity against different pests (Regnault-Roger et al., 2012; de Oliveira et al., 2014; Singh et al., 2021). According to Moustafa et al. (2021), the main compounds

Table 1: Total phenolic and flavonoid content for the four plant’s essential oils

Plant Essential Oils	Total Phenolic Content (mg GAE/g)	Total Flavonoid Content (mg QE/g)
Lemongrass	5.2 ± 0.68 <sup>b</sup>	0.6 ± 0.05 <sup>b</sup>
Gelam	12.3 ± 0.3 <sup>6a</sup>	10.8 ± 0.0 <sup>3a</sup>
Beach vitex	3.4 ± 0.30 <sup>b</sup>	0.4 ± 0.10 <sup>b</sup>
Pandan	1.2 ± 0.45 <sup>c</sup>	0.3 ± 0.0 <sup>6b</sup>

Note: Different alphabet after the value indicates a statistically significant difference (n = 3,  $p < 0.05$ ) among the essential oils with similar phytochemicals content.

of lemongrass leaf EO were  $\alpha$ -citral,  $\beta$ -citral, 5-octyldihydro-2(3H)-furanone, nerylacetal, and trans-verbenol. In particular, citral is considered the key contributor to the insecticidal properties of lemongrass EO (Solomon *et al.*, 2012; Eden *et al.*, 2020). The highest chemical compounds found in gelam leaves were caryophyllene,  $\alpha$ -terpinolene,  $\alpha$ -humulene,  $\beta$ -elemene, and  $\gamma$ -terpinene (Sharif *et al.*, 2019). Casticin is the major flavonoid found in the beach vitex which has many health benefits. In addition, caustic acid, vanillic acid, and 4-hydroxybenzoic acid are some of the phenolic compounds in beach vitex (Azizul *et al.*, 2021). On the other hand, pandan mainly consists of pyranone; coumarins; 1,4-di-tert-butylphenol; pinane; ethyl palmitate; 3,6,6-trimethyl-1-(1-phtalaziny)-1,5,6,7-tetrahydro-4H-indazol-4-one; phytol; purpurogallin; squalene; and decamethyltetrasiloxane (Zakaria *et al.*, 2020).

Table 2 shows the number of prick marks produced by the RPWs after the treatment with the EOs. The long rostrum of the RPW creates prick marks to feed on the substrate or host plant. Therefore, their quantity indicates how much the RPW directly consumed its meal. By making the treated materials unappealing or undesirable, EOs can prevent or disrupt RPW feeding, causing the weevils to remain there for an extended time before starving to death (Talukder *et al.*, 2004; Yappalla *et al.*, 2012). Based on the findings, the plant EOs can be ranked in order of efficacy as follows: Lemongrass > gelam > beach vitex. On the other hand, pandan did not show any antifeedant effect on the RPW (Table 2). Lemongrass EO was proven in many studies to have the highest efficacy in terms of antifeedant among other EOs. For example, lemongrass EO showed the highest feeding deterrent activity against various

Table 2: Mean number of prick marks recorded for five days after treatments with essential oils from four plants compared to control

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5
Control	5.1 ± 0.68 <sup>b</sup>	10.1 ± 0.82 <sup>b</sup>	16.37 ± 1.39 <sup>b</sup>	22.9 ± 1.59 <sup>b</sup>	28.7 ± 1.80 <sup>b</sup>
5% Lemongrass	2.3 ± 0.40 <sup>a</sup>	5.8 ± 0.96 <sup>a</sup>	11.3 ± 1.90 <sup>ab</sup>	16.5 ± 2.19 <sup>a</sup>	18.7 ± 2.18 <sup>a</sup>
10% Lemongrass	2.4 ± 0.44 <sup>a</sup>	6.1 ± 1.07 <sup>a</sup>	9.4 ± 1.28 <sup>a</sup>	14.5 ± 1.64 <sup>a</sup>	17.9 ± 1.8 <sup>a</sup>
	F = 9.26; p < 0.05	F = 6.35; p < 0.05	F = 5.47; p = 0.008	F = 5.95; p = 0.005	F = 9.77; p < 0.05
Control	5.1 ± 0.68 <sup>b</sup>	10.1 ± 0.82 <sup>b</sup>	16.37 ± 1.39 <sup>b</sup>	22.9 ± 1.59 <sup>b</sup>	28.7 ± 1.80 <sup>b</sup>
5% Gelam	2.6 ± 0.40 <sup>a</sup>	7.1 ± 1.33 <sup>ab</sup>	12.3 ± 1.99 <sup>a</sup>	19.0 ± 2.63 <sup>a</sup>	21.5 ± 2.70 <sup>a</sup>
10% Gelam	0.9 ± 0.32 <sup>a</sup>	6.1 ± 1.15 <sup>a</sup>	12.5 ± 2.33 <sup>a</sup>	19.0 ± 3.29 <sup>a</sup>	24.2 ± 4.28 <sup>a</sup>
	F = 18.21; p < 0.05	F = 3.54; p = 0.038	F = 1.45; p = 0.246	F = 0.83; p = 0.442	F = 1.53; p = 0.23
Control	5.1 ± 0.68 <sup>b</sup>	10.1 ± 0.82 <sup>b</sup>	16.37 ± 1.39 <sup>b</sup>	22.9 ± 1.59 <sup>b</sup>	28.7 ± 1.80 <sup>b</sup>
5% Beach vitex	3.6 ± 0.64 <sup>ab</sup>	8.6 ± 0.97 <sup>ab</sup>	14.8 ± 1.70 <sup>a</sup>	22.4 ± 1.83 <sup>a</sup>	25.9 ± 1.63 <sup>a</sup>
10% Beach vitex	1.5 ± 0.26 <sup>a</sup>	6.2 ± 1.06 <sup>a</sup>	13.5 ± 2.08 <sup>a</sup>	23.1 ± 2.90 <sup>a</sup>	27.0 ± 3.03 <sup>a</sup>
	F = 8.02; p = 0.001	F = 4.30; p = 0.02	F = 0.70; p = 0.505	F = 0.28; p = 0.972	F = 0.42; p = 0.661
Control	5.1 ± 0.68 <sup>b</sup>	10.1 ± 0.82 <sup>b</sup>	16.37 ± 1.39 <sup>b</sup>	22.9 ± 1.59 <sup>b</sup>	28.7 ± 1.80 <sup>b</sup>
5% Pandan	3.5 ± 0.5 <sup>5a</sup>	6.7 ± 0.86 <sup>a</sup>	11.2 ± 0.87 <sup>a</sup>	18.2 ± 0.95 <sup>a</sup>	23.5 ± 1.26 <sup>a</sup>
10% Pandan	3.97 ± 0.7 <sup>3a</sup>	8.7 ± 1.53 <sup>a</sup>	13.9 ± 1.66 <sup>ab</sup>	19.8 ± 2.58 <sup>a</sup>	25.9 ± 3.67 <sup>a</sup>
	F = 1.56; p = 0.221	F = 2.42; p = 0.102	F = 3.73; p = 0.032	F = 1.81; p = 0.176	F = 1.22; p = 0.306

Note: Different alphabet indicates statistically significant differences (p < 0.05) among each group.

agricultural insect pests, including *Agrotis ipsilon*, *Callosobruchus maculatus*, *Sitophilus granarius*, and *Phthorimaea operculella* (de Souza Alves et al., 2019; Plata-Rueda et al., 2020; Moustafa et al., 2021). A study by Ibrahim et al. (2017) found that EO derived from lemongrass exhibits significantly higher antifeedant activity against the giant apple snail than EO from betel.

A study by Roszaini et al. (2013) stated that gelam EO was one of the good candidates as an antifeedant for termite control. Likewise, gelam EO also showed feeding inhibition criteria against the brown rice grasshopper (Mardiningsih & Rohimatun, 2021). However, due to its antibacterial and antioxidant qualities, gelam EO was much more well-known in traditional medicines than as an insecticide (Al-Abd et al., 2015). Beach vitex has not been extensively studied for its potential use as an insecticide or antifeedant. However, it is well-known for its antioxidative, antiproliferative, anti-aging properties, and potential as a chemo preventive agent (Rani & Sharma, 2013). It can treat gastrointestinal illnesses, headaches, and inflammation (Azizul et al., 2021). However, other species of *Vitex*, for example, *Vitex negundo* L, exhibited antifeeding behaviour against the cotton leafworms (Shewale et al., 2022) and cotton aphids (Jiang et al., 2016). Among the four plants that were chosen, only pandan EO did not demonstrate any antifeedant effect as evidenced by the fact that the number of prick marks on sugarcane treated with it (either 5% or 10%) did not significantly decrease from the control treatment (Table 2). However, pandan has been proven to possess other insecticidal activities such as larvicidal (Mohamad et al., 2021) and deterrent (Ahmad et al., 1995) on other insect species.

The wore-off antifeedant effect of gelam and beach vitex EO during the third day (Table 2) was a concern, possibly because EOs were completely volatilised or adult RPWs adapted themselves toward EOs through a detoxifying mechanism after feeding on it. Theoretically, a dwindling effect over time and concentration

should be expected. In the vapour phase, EO was vaporised slowly by an air current in a ventilated condition and it could not control the volatile properties of EO (Govere et al., 2000; Oyedele et al., 2002). However, the vapour phase EO can still perform the function in a different mode of action, where gustatory approaches switch to odour modulation (Lee, 2018). Next, RPW is believed to degrade these EOs through detoxification internally. AlJabr et al. (2017) revealed that cytochrome P450, glutathione S-transferase, and esterase in RPW can detect and break down EOs to a lower-level toxic material. Thus, the RPWs were competent to feed on gelam and beach vitex EOs. Further investigation such as odour disruption and RPW detoxification methods should be conducted to understand this issue better.

Table 3 shows the number of RPWs that responded to the chamber and the intensity of reaction (% IR) of the four EOs studied. In general, of the four EOs, gelam and pandan can be considered repellent against RPW with a < 1% IR. On the other hand, lemongrass and beach vitex are weakly attractive (i.e., IR between 1% and 10%). According to Bedini et al. (2019) as cited in Zeni et al. (2021), an EO may have attractant, repellent, or toxic properties dose-dependently. In addition, the shifting of EO's properties from attractant to repellent and finally toxic can be influenced by the species being treated, the EO's composition as well as the EO's concentration (Zeni et al., 2021). Insects such as RPW have highly developed olfactory systems that allow for the detection of and reactions to a variety of volatile cues in the environment. Volatile chemical or odorant detection that results in a certain behaviour mostly entails the reception of odorants in peripheral areas, signal processing in the antennal lobes, and additional processing in the brain (Pellegrino & Nakagawa, 2009; Leal, 2013). Thus, the effects of repellents and attractants in insects depend on how the sensory system detects and processes odour components, with olfactory receptors and neurons being the primary level of interaction (Verschut et al., 2019).

Table 3: Percentage intensity of reaction (% IR) of 10% EOs against the RPW (n = 100)

Essential Oils	Percentage Intensity of Reaction (%)					
	Male		Female		Both	
Lemongrass	8.3	Weakly attractive	-2.2	Repellent	3.2	Weakly attractive
Gelam	-13.0	Repellent	-20.0	Repellent	-16.0	Repellent
Beach vitex	13.6	Attractive	-2.0	Repellent	5.4	Weakly attractive
Pandan	-10.0	Repellent	9.1	Weakly attractive	0.0	Repellent

In a study conducted on sweet potato weevils, Samantaray and Korada (2016) observed that some compounds such as cis-3-hexen-1-ol, consistently elicited high axonal action potentials within the olfactory receptors of both genders while cis-3-hexanal and  $\alpha$ -humulene elicited the highest potentials in female antennal receptors relative to male weevils. These compounds exhibited a repellent behaviour, resulting in the reduced attraction of weevils. This indicates that  $\alpha$ -humulene is one of the key chemical constituents found in gelam (Sharif *et al.*, 2019), the repellent behaviour of RPWs as shown in Table 3. On the other hand, pandan leaves were the most effective in repelling rice weevils, *S. oryzae* with a repellency percentage of 47% among other plants studied (Mohd Arafah *et al.*, 2023).

Gender wise, the female RPWs reacted negatively to three of the four EOs studied (Table 3). Contrastingly, the male RPWs were attracted to lemongrass and beach vitex EOs but were repelled by gelam and pandan EOs. The differences in the behaviour between the male and female RPWs observed may be due to several factors. Male and female insects can possess differences in their sensory organs. For example, male RPWs have reddish-brown hairs on their elongated snout or rostrum while females have no hairs and are longer, slender, and slightly downward-curvature (Abdel-Banat & El-Shafie, 2023). The differences in olfactory receptors or sensitivity might lead to varying responses to volatile repellents. In addition, it is also possible that male and female weevils

have distinct nutritional requirements, which could affect how they react to EOs. In contrast to females, which may have varied preferences due to their particular requirements during egg-laying or the development of progeny, males may be more drawn to or repelled by certain plant compounds that supply vital nutrients for their reproductive activities (Genç, 2006; Wu *et al.*, 2020; Huck *et al.*, 2021). The physiology and genetic makeup of male and female weevils differ, which has an impact on behaviour. These discrepancies may be caused by different hormone levels (Nijhout & Laub, 2018) or the expression of specific genes (Weitekamp *et al.*, 2017). As a result, compared to females, the EOs may cause different physiological or neurological reactions in males, resulting in divergent behaviours (Krzyżowski *et al.*, 2020).

EOs that are considered attractants can also be beneficial in sustainable pest management. The attractive EOs can be employed in conjunction with the biological control agents. They can attract pests into an area where natural predators have been released (Liu *et al.*, 2019). Certain EOs have an appealing quality that attracts insects who use them as aggregation signals. For example,  $\alpha$ -copaene, a minor component in the EOs of various plant species is a potent attractant for the male Mediterranean fruit flies, *Ceratitidis capitata* and potentially serves as a chemical cue for the female flies to aggregate with the male flies during mating (Nishida *et al.*, 2000). Furthermore, an environmentally friendly method of pest control can be achieved by reducing the total reliance

on chemical pesticides through the use of attractants in pest management.

As stated earlier, phenolic and flavonoid compounds play an important role in the insecticidal activities of EOs. The phenolic compounds from EO are believed to play a crucial role in chemical defence mechanisms, as they often carry out antifeeding, digestibility-reducing, and other effects on herbivores or insects (Rehman *et al.*, 2012; Pratyusha, 2022). In this study, gelam EO contained the highest amount of TPC and TFC (Table 1), yet it was not the most effective EO in reducing the consumption rate of adult RPWs. Contrastingly, pandan oil with the lowest TPC and TFC content (Table 1) showed a repellent activity against the male RPWs but no antifeedant activity. Perhaps not surprisingly, while containing a high amount of phenolic compounds, a certain chemical constituent is the most important part that plays a role in the defence against a specific pest. For example, citral and genaryl acetate from lemongrass EO caused toxic and repellent effects on *Anticarsia gemmatilis* (Brügger *et al.*, 2021). On the other hand, filiferol on the other hand, was found to be involved in defence against *Rhynchophorus ferrugineus* in *Washingtonia filifera* palm (Cangelosi *et al.*, 2015). These studies pointed out that the defence mechanism of an insect often took effects from a specific chemical compound but not the overall amount of phenolic compounds. In addition, pandan EO contains some compounds that insects find repellent, but possibly in lower concentrations or different types than those in gelam, lemongrass, and beach vitex. Therefore, it was insufficient to correlate the TPC and TFC of EO with its antifeedant and repellent properties; nonetheless, these values were still significant for recording and determining other bioactivities.

## Conclusions

This study offers strong proof of the effectiveness of EOs obtained from lemongrass (*C. citratus*), gelam (*M. cajuputi*), beach vitex (*V. rotundifolia*), and pandan (*P. amaryllifolius*)

as possible bioinsecticides against the invasive RPW. The results show that these natural oils have different levels of antifeedant and repellent properties, which are important for creating environmentally friendly pest management strategies. With the highest concentrations of phenolics and flavonoids, gelam oil stood out and may have significant insecticidal potential. It demonstrated notable repulsive qualities against RPWs, as did pandan oil. On the other hand, lemongrass oil showed the strongest antifeedant efficacy but with a noticeably reduced repelling impact. The study also found that different genders responded differently to these oils, underscoring the significance of considering gender when developing pest management plans. Except for pandan, female RPWs were generally more vulnerable to the repelling properties of the oils, whereas male RPWs exhibited clear preferences being drawn to beach vitex and lemongrass but repulsed by gelam and pandan.

These results provide insightful information for the agriculture industry, especially in areas with problematic RPW infestations. These EOs provide an eco-friendly substitute for traditional chemical pesticides, supporting the larger goals of sustainable agriculture and natural resource management. Subsequent investigations may build upon these results by investigating the molecular underpinnings of these oils' insecticidal characteristics and evaluating their usefulness in real-world settings. This study also highlights the potential of natural resources in resolving agricultural difficulties and adds to the expanding body of knowledge supporting the use of chemicals derived from plants in integrated pest management.

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### Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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