PREPRINT

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Evaluation of Fermented Plant Extracts as Bioinsecticides in Controlling *Phenacoccus solenopsis* Colonies on *Hibiscus rosa-sinensis* under Laboratory Conditions

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ABSTRACT

The mealybug, *Phenacoccus solenopsis*, is a serious pest of *Hibiscus rosa-sinensis*. The waxy coating on its body may hinder pesticide penetration, and the extensive use of pesticides is risky to humans and the environment. Considering these drawbacks, fermented plant extracts (FPEs) were explored for their potential use in controlling this pest in a more user and environmentally-friendly manner. FPEs derived from eleven plant materials were evaluated against *P. solenopsis* for their insecticidal activity, mealybug wax removal potential and phytotoxicity effect on *H. rosa-sinensis*. Five concentrations of FPE [5, 10, 15, 20, and 25% (w/v)] were prepared. Among the 11 FPEs, FPE derived from ficus, kaffir lime, and turmeric were effective in suppressing *P. solenopsis* with lethal concentration at 50% (LC_{50} value) less than 20% concentration. Although ficus FPE was the top performer in the insecticidal assay, it induced medium to very high levels of leaf damage after being treated with 15–25% concentration at 24 and 72 hr post-treatment. A low level of leaf damage was observed in treatment with turmeric and kaffir lime FPEs at 72 hr post-treatment. FPEs could remove wax from the body of *P. solenopsis* with no significant difference among them. In conclusion, the FPE of ficus, kaffir lime, and turmeric showed promising insecticidal effects against *P. solenopsis*.

Keywords: Fermented plant extract, *Hibiscus rosa-sinensis*, *Phenacoccus solenopsis*, phytotoxicity

ARTICLE INFO

Article history:
Received: 07 August 2023
Accepted: 18 September 2023
Published: 17 April 2024

DOI: https://doi.org/10.47836/pjtas.47.2.03

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ISSN: 1511-3701
e-ISSN: 2231-8542

INTRODUCTION

Phenacoccus solenopsis is a destructive polyphagous pest that has infested over 154 plant species belonging to 53 families, including 25 shrubs and trees, 20 field and horticultural crops, 45 ornamentals, and 64 weeds (Alam et al., 2011). *Phenacoccus solenopsis* reproduces sexually and completes many generations in a year. It has been reported as a serious pest of *Hibiscus rosa-sinensis* in Pakistan, India, Nigeria, and Malaysia (Al-obaidy et al., 2017). It was recorded for the first time on *H. rosa-sinensis* in Malaysia in 2016 (Sartiami et al., 2016). The infested *H. rosa-sinensis* showed malformation of buds, shortened twigs and branches, stunted shoots, or wrinkled young leaves. *Phenacoccus solenopsis* did not cause severe economic loss in the country. However, it incurred 1.3 million bales of losses in cotton production in Pakistan (Abdullah, 2009) and 14.9–53.6% yield losses in India (Kumar et al., 2014). It is a threat to agricultural and horticultural industries. Synthetic chemicals are effective for the control of *P. solenopsis*. However, the frequent and indiscriminate application of chemical insecticides has led to a resurgence of new pests, insects resistant to insecticides (Omarini et al., 2020), destruction of beneficial non-target organisms (parasitoids and predators), hazardous effects on human beings, food and water contamination, and negative impact on biological diversity (Khaliq et al., 2012). The waxy coating on their body may hinder the penetration of insecticides and make it challenging to manage the mealybugs efficiently (Badshah et al., 2015). Considering these drawbacks, alternate options need to be explored to control this pest in a more user-friendly and environmentally friendly manner.

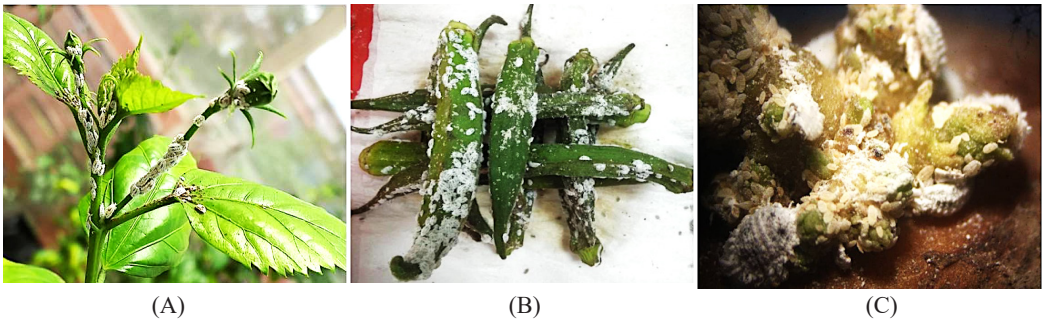
Fermented plant extract (FPE) has been evaluated on aphids, whiteflies (Nzanza & Mashela, 2012), 28-spotted beetles (Baloc & Bulong, 2015), cutworm (Sahayaraj et al., 2011), mosquitoes, flies, rats, cockroaches (Neupane & Khadka, 2019), and citrus mealybug (Khadem et al., 2022). In India, FPEs were used to control the pests of onion, brinjal, and sugarcane occasionally (Sahayaraj et al., 2011). They are produced by natural fermentation of plant materials with sugar and water. It is believed that the byproducts of FPE, which consist of acetic acid, "vinegar", alcohol, and propionic acid, contain insecticidal potentiality (Nazim & Meera, 2017; Neupane & Khadka, 2019). These byproducts seem to provide a commercial option to replace conventional chemical control and could overcome insecticide resistance. FPE has been proven to cause mortality in *Planococcus citri* under laboratory conditions and to be able to remove wax from the body of *P. citri* (Khadem et al., 2022). However, Nazim and Meera (2017) reported that the byproducts of FPE, such as acetic acid, "vinegar", alcohol, and propionic acid, could induce a phytotoxicity effect on plant leaves. The severity of the phytotoxicity effect of a particular FPE depends on the plant material used in preparing the FPE. There is no report on the potential use of FPE against *P. solenopsis*. Therefore, this study was conducted to evaluate the

insecticidal potential of different FPEs against *P. solenopsis* as well as their wax removal efficiency on *P. solenopsis*. Since the byproduct of FPEs may cause certain degrees of leaf damage to the test plant, the phytotoxicity level of different FPEs was conducted on *H. rosa-sinensis* to ensure optimum use of FPEs for effective control of *P. solenopsis*.

MATERIALS AND METHODS

Insect

Phenacoccus solenopsis were collected from infested *H. rosa-sinensis* in the surroundings of Universiti Putra Malaysia (UPM, latitude of 03°00 N′, longitude 101°72′ E and altitude of 64 m a.s.l.), Selangor, Malaysia. Mealybug specimens were collected under field conditions at 28–32°C and 60–65% relative humidity. Infested twigs and leaves were brought to the Laboratory of Insect Pathology, UPM, for mass rearing. Green okra B501 and sprouted Granola potatoes were used for rearing *P. solenopsis* (Badshah et al., 2015). They were purchased from the local markets and dipped into 0.1% (v/v) sodium hypochlorite (Clorox®, Malaysia) for 10 min, followed by washing with distilled water (dH_2O) and air drying at room temperature. Okra and potatoes were put into plastic aquariums after air drying. The potatoes were sprinkled with water daily to encourage sprouting. When the sprouts reached 5–7 cm in length, they were used for rearing *P. solenopsis*. The females of *P. solenopsis* were collected gently from the infested plant parts of *H. rosa-sinensis* with a camel hairbrush and then released onto the sprouted potatoes and green okra to generate an F_1 population (Figure 1). The mealybugs were maintained at room temperature of 25±2°C and relative humidity (RH) of 65±5%.



(A) (B) (C)

Figure 1. *Phenacoccus solenopsis* sampled from infested (A) *Hibiscus rosa-sinensis*, (B) reared on green okra and (C) sprouted potatoes

Plant Materials and Fermentation

A total of 11 plant species, namely peppermint (*Mentha piperita*), Mexican mint (*Plectranthus amboinicus*), variegated mint (*Plectranthus madagascariensis*), onion bulb (*Allium cepa*), turmeric rhizome (*Curcuma longa*), aromatic ginger (*Kaempferia galanga*), kaffir lime (*Citrus hystrix*), lime (*Citrus aurantiifolia*), garlic (*Allium sativum*), mahogany (*Swietenia*

mahagoni), and ficus (*Ficus hispida*) were used in this study (Table 1). These plant materials were selected based on their insecticidal activity and availability in the study area. Peppermint, onion bulb, turmeric rhizome, kaffir lime, lime, and garlic were purchased from the local markets located nearby UPM. Other plants were cultivated in UPM. Plant materials were subjected to a 5 min surface sterilisation with 0.1% (v/v) sodium hypochlorite (Clorox®, Malaysia), followed by rinsing twice with dH_2O and air-drying at room temperature. The plant materials were chopped with a sterile sharp knife on a chopping board, placed into airtight plastic containers, and mixed with molasses and dH_2O at 3:1:10 (w:v:v). A quarter of the container was kept vacant, and the lid was closed tightly. The containers were stored in a dry, cool, and shady place. The lid of the containers was untightened once a week to release the air generated in the containers during fermentation. The containers were shaken gently every week. After three months of fermentation, the FPEs were filtered with a Whatman No. 1 filter paper placed on a Falcon® 50 ml high-clarity polypropylene centrifuge tube. The filtrate was kept at -80°C overnight prior to freeze-drying at -110°C for approximately 3 days. The filtrate was then stored at -20°C.

Table 1
List of plant materials used in the preparation of fermented plant extracts

Common name	Scientific name	Family	Part	References
Peppermint	*Mentha piperita*	Lamiaceae	Leaf	Karamaouna et al. (2013)
Variegated mint	*P lectranthus madagascariensis*	Lamiaceae	Leaf	Matias et al. (2019)
Mexican mint	*Plectranthus amboinicus*	Lamiaceae	Leaf	Arumugam et al. (2016)
Onion	*Allium cepa*	Lilliaceae	Bulb	Gharsan et al. (2018)
Turmeric	*Curcuma longa*	Zingiberaceae	Rhizome	de Souza Tavares et al. (2016)
Aromatic ginger	*Kaempferia galanga*	Zingiberaceae	Leaf	Liu et al. (2014)
Lime	*Citrus aurantiifolia*	Rutaceae	Fruits	Bilal et al. (2012); Karamaouna et al. (2013)
Kaffir lime	*Citrus hystrix*	Rutaceae	Fruits	Loh et al. (2011)
Garlic	*Allium sativum*	Lilliaceae	Clove	Okolle et al. (2018)
Mahogany	*Swietenia mahagoni*	Meliaceae	Leaf	Yasmin et al. (2017)
Ficus	*Ficus hispida*	Moraceae	Leaf	Ahmadi et al. (2012)

Insecticidal Assay

The insecticidal effect of FPEs was tested against the third instar nymph of *P. solenopsis* at the Laboratory of Insect Pathology, UPM, following the method of Mostafa et al. (2018) with some modifications. Petri dishes of 9 cm diameter were used in this experiment. The chemical-free, disease-free, and non-infested leaves of *H. rosa-sinensis* were collected and disinfected prior to the experiment. Leaves with a diameter of 9 cm were selected, and the petiole of each leaf was wrapped with wet cotton wool and aluminium foil to keep the leaves turgid for a longer period. A single leaf was placed in a Petri dish. A moist filter paper was put under the leaf in each Petri dish. A total of 10 *P. solenopsis* (third instar) were released onto each leaf with a soft camel hairbrush. The FPE was diluted with distilled water into 5, 10, 15, 20, and 25% (w/v) using the $C_1V_1 = C_2V_2$ formula where C_1 and C_2 are the concentration of the first and second solution, and V_1 and V_2 are the volume of the first and second solution, respectively (Khadem et al., 2022).

The FPEs were applied to the mealybugs and left with a hand atomiser (250 µl/replication). Treatment with dH_2O served as the negative control. A commercial botanical insecticide (A-Force, Malaysia) containing 0.1% citrus extract and aqua solvent was used as the positive control. After spraying, the Petri dishes were covered with a fine muslin cloth to provide ventilation to *P. solenopsis* and prevent them from escaping. The petri dishes were then covered with a lid. The bioassay was performed under a completely randomised design (CRD) with 10 replications, maintained under temperature at 25±2°C and relative humidity of 65±5%. The mortality of *P. solenopsis* was recorded at 24, 48, 72, 96, and 120 hr post-treatment. *Phenacoccus solenopsis* was considered dead if they did not move their legs after gentle probing with a camel hairbrush for 2 s under Dino-Lite eye microscope 2.0 (Ganjisaffar et al., 2019). The percentage of mortality was calculated using the following formula (Henderson & Tilton, 1955):

$$\% \text{ Corrected mortality} = (1 - T_a \times C_b / T_b \times C_a) \times 100\% \quad [1]$$

where T_a is the number of alive insects after treatment in the treated area, T_b is the number of alive insects before treatment in the treated area, C_a is the number of alive insects after treatment in the control area, and C_b is the number of alive insects before treatment in the control area.

Phytotoxicity Assay

The phytotoxicity test used chemical-free, disease-free, and non-infested *H. rosa-sinensis* leaves. Leaves were rinsed with 0.1% (v/v) sodium hypochlorite (Clorox®, Malaysia) for 5 min, followed by rinsing with dH_2O twice and then air-dried prior to the experiment. Each leaf petiole was wrapped with wet cotton wool and aluminium foil to keep the leaf turgid during the experiment. The leaves were dipped in five different concentrations [5, 10, 15, 20, and 25% (w/v)] of FPEs for 30 s, and the negative control

was treated with distilled water only. For each concentration, ten replications were used, and the treated leaves were observed for the presence of leaf discolouration or formation of necrotic spots on the leaf at 24 and 72 hr post-treatment. Equation 2 calculated the percentage of damaged leaves (Sreerag & Jayaprakas, 2014). The severity of the leaf damage was graded using the rating scale in Table 2.

Table 2
Phytotoxicity rating scale

Level	% Leaf damage*	Severity
0	<1	No
1	1–10	Low
2	11–20	Moderate
3	21–30	High
4	31–40	Very high
5	41–50	Severe
6	>50	Very severe

Note. *The leaf damage percentage was calculated based on the damaged area over the total surface leaf area x 100%

Mealybug Wax Removal Test

The experiment was carried out in CRD with ten replications under laboratory conditions. At first, 10 adults of *P. solenopsis* of similar body size were weighed (weight before treatment = W_0) for each treatment. A hand atomiser sprayed an equal volume of FPEs (250 µl/replication) on the adults. Mealybugs sprayed with dH_2O served as a negative control, while mealybugs sprayed with 100% (v/v) chloroform (Sigma-Aldrich, USA) served as a positive control. At 72 hr post-treatment, the body weight of adults (W_T) was taken. The adults were then dipped in 100% (v/v) chloroform (Sigma-Aldrich, USA) for 60 s in a beaker to harvest the remaining undetached wax on the body of *P. solenopsis*. A syringe filter removed the dirt in the dissolved wax. The dissolved wax was harvested by drying at 37°C for 24 hr in an incubator (Salunkhe et al., 2013). The weight of the dissolved wax (W_{DW}) was measured. The amount of detached mealybug wax was calculated using Equation 3.

where W_0 is the weight of mealybugs before treatment, W_T is the weight of mealybugs after treatment, and W_{DW} is the weight of dissolved wax extracted from FPE-treated mealybugs by chloroform.

Statistical Analysis

Recorded data were subjected to analysis of variance using the statistical program SAS® software (version 9.4) and means comparison using Tukey's studentised range test. The LC_{50} value of each FPE was determined using POLO-Plus (version 0.03).

RESULTS AND DISCUSSION

Insecticidal Assay

The present study demonstrated the insecticidal activity of 11 FPEs derived from peppermint (*M. piperita*), Mexican mint (*P. amboinicus*), variegated mint (*P. madagascariensis*), onion bulb (*A. cepa*), turmeric rhizome (*C. longa*), aromatic ginger (*K. galanga*), kaffir lime (*C. hystrix*), lime (*C. aurantiifolia*), garlic (*A. sativum*),

mahogany (*S. mahagoni*), and ficus (*F. hispida*) against *P. solenopsis* (Table 3). The result revealed that the mortality of *P. solenopsis* was dependent on FPE concentration and exposure time. There was a gradual increment in the mortality rate of *P. solenopsis* with an increase in the FPE concentration and exposure time. The mortality rate of *P. solenopsis* induced by FPEs was lower than that of A-Force at 5% concentration at 72 hr post-treatment. When the concentration of FPE was increased to 10%, only kaffir lime FPE (42% mortality) was comparable to A-Force treatment (45% mortality). The highest mortality of *P. solenopsis* was observed with ficus FPE (61–74%), while the lowest mortality was observed with garlic FPE (16–28%) at 15–25% FPE concentrations. The onion, turmeric, and kaffir lime FPEs exhibited a higher mortality rate than A-Force at 72 hr post-treatment. The exposure time was further increased to 120 hr, and higher mortality of *P. solenopsis* was recorded in all FPE treatments. Other FPEs, such as mahogany, onion, Mexican mint, and variegated mint, performed better than A-Force at 120 hr post-treatment. FPEs such as peppermint, garlic, lime, and aromatic ginger were not comparable to the A-Force in the *P. solenopsis* control.

The LC_{50} value towards *P. solenopsis* was 12.89% for ficus FPE, followed by 12.87% for kaffir lime FPE, 18.12% for turmeric FPE, 22.23% for onion FPE, 23.99% for mahogany FPE, 30.78% for Mexican mint FPE, 31.38% for variegated mint FPE, 57.85% for peppermint FPE, 68.46% for aromatic ginger FPE, 87.40% for lime FPE, and the least efficacious FPE was garlic (93.82%) recorded at 72 hr post-treatment (Table 4). Similar results were recorded at 120 hr post-treatment, except the onion and peppermint FPEs were less effective when exposed for a longer time to *P. solenopsis*. Among the FPEs tested, ficus FPE exhibited the highest insecticidal action against *P. solenopsis* throughout the exposure time compared to other FPEs. *Ficus* sp. has a broader spectrum of insecticidal and acaricidal properties (Romeh, 2013). It has been proven effective against the fourth instar larvae of *Aedes albopictus* (Wang et al., 2011). The fermented form of ficus showed a very strong and promising insecticidal action to *P. solenopsis* in the present study.

Kaffir lime and lime belong to the Rutaceae family and genus *Citrus*. The essential oil of citrus contains insecticidal properties against *P. ficus* (Karamaouna et al., 2013) and *Spodoptera litura* (Loh et al., 2011). Besides citrus fruit, citrus seed extracts, such as *C. sinensis* and *C. reticulata* (L.), were also effective against *Ae. albopictus* (Bilal et al., 2012). The fermented form of kaffir lime caused significant mortality to *P. solenopsis* in the present study. The results are consistent with those reported by Khadem et al. (2022) that *P. citri* exposed to kaffir lime FPE exhibited a high mortality rate. Although lime belongs to the genus *Citrus*, the fermented form of lime did not show promising control on *P. solenopsis* in the present study. It required more than 6 times higher FPE concentration

than kaffir lime FPE to cause 50% mortality in *P. solenopsis* at 72 hr post-treatment. Khadem et al. (2022) also reported poor insecticidal activity of fermented lime extract against *P. citri*.

The essential oil of turmeric contains pesticidal activity against insects and weeds (de Souza Tavares et al., 2016). Natural compounds such as arturmerone could induce insect mortality (de Souza Tavares et al., 2016). In the present study, the insecticidal activity of turmeric FPE is comparable with those of ficus and kaffir lime which required less than 20% FPE concentration to kill 50% mealybugs at 72 hr post-treatment.

In the present study, onion FPE revealed its capability of inducing high mortality in *P. solenopsis*. Other mealybugs, such as *P. citri*, were also reported to be susceptible to onion FPE (Khadem et al., 2022). Onion, in the form of essential oil, had shown its potential insecticidal activity against *Ae. aegypti* (Susheela et al., 2016) and *Oryzaephilus surinamensis* (Gharsan et al., 2018). Some previous studies have confirmed that 10% aqueous solution of onion oil could inhibit the hatching of embryonated eggs and induce acaricidal activity in all stages of *Boophilus annulatus* at more than 5% (v/v) concentrations (Aboelhadid et al., 2013).

Mahogany has been proven effective in controlling aphids in essential oil (Yasmin et al., 2017) and termites in the form of leaves, seeds, and bark extract (Cruz et al., 2018). The mahogany FPE tested in the present study also demonstrated good insecticidal activity against *P. solenopsis*. Other FPEs such as Mexican mint, variegated mint, and peppermint demonstrated 31–46% mortality to *P. solenopsis* at 72 hr post-treatment. They are members of the *Mentha* family. The results of this *Mentha* spp. are consistent with those reported previously that *Mentha* spp. could inhibit the growth and reproduction of some important pests, namely *Planococcus ficus* (Karamaouna et al., 2013), *Ferrisia virgata* (El-Ashram et al., 2020), and *P. citri* (Khadem et al., 2022). Among the *Mentha* spp. tested, Mexican mint and variegated mint FPEs exhibited less than 50% mortality, whereas peppermint FPE caused less than 35% mortality on *P. solenopsis* at 72 hr post-treatment. Khadem et al. (2022) reported that the Mexican mint FPE had the best FPE (LC_{50} = 1.83%) against *P. citri*; however, the Mexican mint FPE was not promising against *P. solenopsis* in the present study. Different insects may have different hydrophilic-hydrophobic cuticle structures that could influence the penetration of an FPE into an insect's body (Alotaibi et al., 2022). Although garlic extract has been demonstrated to be toxic to the long-tailed mealybugs on banana plants (Okolle et al., 2018), the garlic FPE showed the least insecticidal activity to *P. solenopsis* (LC_{50} value higher than 60%) in the present study.

Phytotoxicity Effect of FPEs on *H. rosa-sinensis* Leaves

Results presented in Table 5 indicated different levels of phytotoxicity of FPEs on *H. rosa-sinensis* leaves. Turmeric, kaffir lime, and ficus FPEs were selected for

Table 3
Mortality of Phenacoccus solenopsis *after treatment with fermented plant extracts (FPEs) at 72 and 120 hr post-treatment. Values are expressed in mean ± S.E*

FPE	% Mean mortality of *P. solenopsis*									
	72 hr					120 hr				
	5%	10%	15%	20%	25%	5%	10%	15%	20%	25%
Peppermint	5 ± 1.67^{ef}	15 ± 1.67^{ef}	16 ± 1.63^{g}	23 ± 2.13^{g}	31 ± 2.77^{gh}	11 ± 2.33^{ef}	33 ± 2.60^{cd}	40 ± 2.11^{e}	45 ± 1.67^{e}	53 ± 3.00^{e}
Mexican mint	15 ± 2.23^{de}	21 ± 2.77^{def}	25 ± 2.24^{efg}	45 ± 3.41^{bcd}	46 ± 3.39^{def}	29 ± 3.48^{bcd}	33 ± 3.00^{cd}	44 ± 3.05^{de}	67 ± 3.00^{b}	71 ± 3.14^{c}
Variegated mint	6 ± 2.21^{ef}	20 ± 1.49^{def}	32 ± 1.33^{def}	36 ± 2.21^{def}	40 ± 3.65^{efgh}	12 ± 2.49^{ef}	33 ± 2.13^{cd}	56 ± 2.67^{bcd}	65 ± 2.23^{b}	69 ± 1.79^{cd}
Onion	8 ± 2.00^{ef}	22 ± 2.00^{cdef}	38 ± 2.49^{bcd}	40 ± 2.58^{de}	58 ± 2.91^{bcd}	17 ± 2.13^{de}	29 ± 3.48^{d}	58 ± 2.49^{bc}	62 ± 2.00^{bcd}	71 ± 2.77^{c}
Garlic	9 ± 1.79^{def}	12 ± 1.33^{f}	16 ± 2.21^{g}	22 ± 3.59^{g}	28 ± 3.89^{h}	26 ± 2.21^{cd}	29 ± 1.79^{d}	40 ± 2.98^{e}	42 ± 2.49^{e}	51 ± 3.14^{e}
Turmeric	19 ± 1.79^{cd}	21 ± 2.33^{def}	47 ± 4.23^{bc}	53 ± 3.00^{abc}	63 ± 3.67^{abc}	25 ± 2.68^{cd}	32 ± 4.16^{cd}	59 ± 3.48^{bc}	69 ± 2.33^{b}	72 ± 2.00^{c}
Kaffir lime	32 ± 2.00^{b}	42 ± 2.91^{ab}	48 ± 3.26^{b}	56 ± 2.21^{ab}	66 ± 2.21^{ab}	35 ± 2.23^{bc}	49 ± 3.48^{ab}	62 ± 2.49^{b}	74 ± 3.05^{a}	86 ± 3.39^{ab}
Lime	10 ± 1.49^{def}	22 ± 1.33^{cdef}	22 ± 1.33^{fg}	23 ± 1.52^{g}	28 ± 2.00^{h}	28 ± 3.26^{bcd}	38 ± 2.49^{bcd}	43 ± 2.60^{e}	50 ± 2.11^{de}	57 ± 1.53^{de}
Aromatic ginger	12 ± 2.00^{de}	16 ± 2.21^{ef}	23 ± 2.13^{fg}	25 ± 1.67^{fg}	39 ± 5.04^{efgh}	22 ± 3.59^{de}	35 ± 2.68^{bcd}	43 ± 2.60^{e}	51 ± 3.14^{cde}	57 ± 3.35^{de}
Mahogany	11 ± 3.14^{de}	32 ± 2.49^{bc}	36 ± 2.67^{cde}	43 ± 3.00^{cd}	51 ± 2.33^{cde}	26 ± 3.39^{cd}	55 ± 3.07^{a}	63 ± 3.00^{b}	64 ± 2.21^{b}	75 ± 1.67^{bc}
Ficus	26 ± 2.20^{bc}	30 ± 2.10^{dc}	61 ± 2.33^{a}	62 ± 2.00^{a}	74 ± 1.63^{a}	40 ± 1.49^{ab}	46 ± 2.67^{abc}	76 ± 3.05^{a}	86 ± 1.63^{a}	90 ± 2.11^{a}
A-Force (Standard)	45 ± 3.07^{a}	45 ± 3.07^{a}	45 ± 3.07^{bc}	45 ± 3.07^{bcd}	45 ± 3.07^{defg}	48 ± 2.00^{a}	48 ± 2.00^{ab}	48 ± 2.00^{cde}	48 ± 2.00^{e}	48 ± 2.00^{e}
Water (control)	0 ± 0^{f}	0 ± 0^{g}	0 ± 0^{h}	0 ± 0^{h}	0 ± 0^{i}	0 ± 0^{f}	0 ± 0^{e}	0 ± 0^{f}	0 ± 0^{f}	0 ± 0^{f}

Note. Mean of ten replications. Original values given in a column mean followed by the same letter are not significantly different at $P < 0.001$ as per Tukey's studentised range test

Table 4
Median lethal concentration (LC_{50}) of fermented plant extracts (FPEs) against third-instar nymphs of Phenacoccus solenopsis

FPE	72 hr post-treatment		120 hr post-treatment	
	LC_{50}* (95% C. L.) (%)	χ^2	LC_{50}* (95% C. L.) (%)	χ^2
Ficus	12.89 (11.175–14.813)	17.87	7.75 (6.49–8.87)	25.17
Kaffir lime	13.87 (11.037–17.832)	13.89	9.02 (7.47–10.42)	24.60
Turmeric	18.12 (15.686–21.769)	26.66	10.16 (8.95–14.37)	24.67
Onion	22.23 (19.279–27.103)	20.04	14.28 (12.68–16.14)	19.20
Mahogany	23.99 (19.770–32.635)	20.65	10.35 (8.56–12.06	18.50
Mexican mint	30.78 (23.789–49.306)	23.67	13.69 (11.68–16.13)	30.16
Variegated mint	31.38 (24.972–46.641)	15.98	14.43 (12.92–16.18)	14.38
Peppermint	57.85 (37.910–149.881)	15.73	21.87 (18.38–28.31)	18.86
Aromatic ginger	68.46 (38.852– 96.331)	17.56	19.20 (15.72–25.88)	19.95
Lime	87.40 (45.201–158.303)	7.57	19.31 (15.0–29.756)	12.67
Garlic	93.82 (47.713–172.101)	23.28	28.73 (20.39–66.11)	14.67

Note. * Lethal concentration killing 50% of the exposed adult population; C. L. = Confidence Level

phytotoxicity study based on their low LC_{50} value in the insecticidal assay. The severity of the leaf damage increased with exposure time and concentration of FPE. Among the FPEs tested, the turmeric and kaffir lime showed low levels of phytotoxicity to *H. rosa-sinensis* leaves after treatment with 20–25% FPE at 72 hr post-treatment. Although ficus FPE showed the highest potential in the insecticidal assay, it induced medium to very high leaf damage after treatment with 15–25% FPE at 24 and 72 hr post-treatment. A lower concentration of ficus and kaffir lime at the proposed LC_{50} value showed a very low percentage of leaf damage (<5%) at 72 hr post-treatment. De Martino et al. (2010) and Vokou et al. (2003) reported that monoterpenes and oxygenated compounds (ketones, alcohols, aldehydes, and phenols) accredited to ficus seem to be responsible for phytotoxicity activity. Khadem et al. (2022) also documented different levels of leaf damage caused by different fermented plant materials on citrus leaves. According to Mousavi et al. (2021), the phytotoxic potential of a plant varies with plant species and the concentration of an extract. The level of leaf damage could be induced by chemical, botanical, or biological pesticides; therefore, a phytotoxicity assessment is needed prior to registering a plant material as a pesticide (Karamaouna et al., 2013). The phytotoxic effect of FPEs on *H. rosa-sinensis* leaves is shown in Figure 2.

Table 5
Phytotoxic effect of fermented plant extracts (FPEs) on Hibiscus rosa-sinensis *leaves*

Treatment	% Leaf damage									
	24 hr					72 hr				
	5%	10%	15%	20%	25%	5%	10%	15%	20%	25%
Turmeric FPE	0^{a} (N)	0^{b} (N)	0^{b} (N)	0^{b} (N)	0^{b} (N)	0^{a} (N)	0^{b} (N)	0.5^{b} (N)	1^{b} (L)	1^{b} (L)
Kaffir lime FPE	0^{a} (N)	0^{b} (N)	0^{b} (N)	0^{b} (N)	0^{b} (N)	0^{a} (N)	0^{b} (N)	0^{b} (N)	0.7^{b} (N)	1^{b} (L)
Ficus FPE	1^{a} (L)	2^{a} (L)	7^{a} (L)	12^{a} (M)	22^{a} (H)	1^{a} (L)	5.5^{a} (L)	12^{a} (M)	32^{a} (VH)	34^{a} (VH)
Water	0^{a} (N)	0^{b} (N)	0^{b} (N)	0^{b} (N)	0^{b} (N)	0^{a} (N)	0^{b} (N)	0^{b} (N)	0^{b} (N)	0^{b} (N)

Note. Mean of ten replications. Original values given in a column mean followed by the same letter are not significantly different at $P < 0.001$ as per Tukey's studentised range test. The value in parenthesis indicates grade of leaf damage: N = No; L = Low; M = Moderate; H = High; VH = Very high

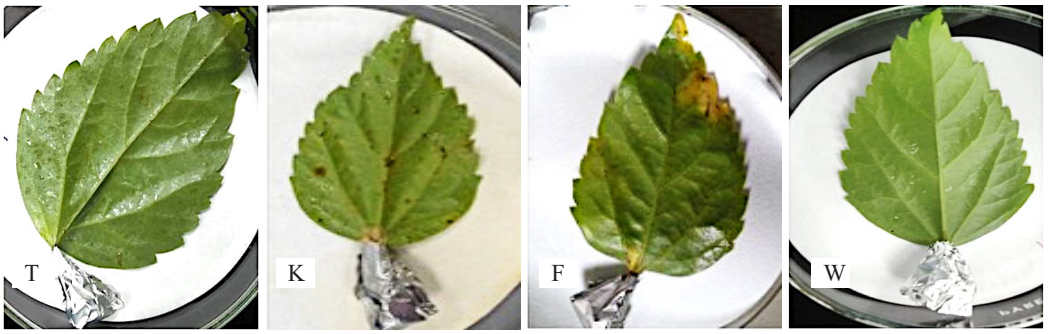


Figure 2. Phytotoxicity effect of fermented plant extract (FPE) on *Hibiscus rosa-sinensis* leaves at 72 hr post-treatment

Note. T = Turmeric FPE, K = Kaffir lime FPE, F = Ficus FPE, W = Distilled water

Effect of FPEs on Mealybug Wax

The turmeric, ficus, and kaffir lime FPEs tested in the present study were able to remove the wax from the body of *P. solenopsis*, along with their insecticidal potential (Figure 3). The percentage of wax removal increased with the concentration of FPEs. There was no significant difference among the test FPEs except the FPE of kaffir lime at 20% concentration. An ascending order of the percentage of mealybug wax removal was observed when higher concentrations of FPE were applied. Overall, FPE of turmeric, kaffir lime, and ficus below 20% could remove less than 50% of mealybug wax (44.16, 33.48, and 41.95%, respectively) compared to 96.58% of mealybug wax removed by chloroform. Chloroform has been reported to dissolve plants' epicuticular wax (Loneman et al., 2017) and mealybug wax (Salunkhe et al., 2013). The FPEs tested in the present study had shown their wax-removing potential in the mealybug wax of *P. solenopsis* (Figure

4). Mealybugs have a waxy layer on their body that protects them from the penetration of insecticides. Removing wax from the insect cuticle could cause dehydration of the membrane cells, which may result in the death of the insects (Regnault-Roger et al., 2012). Consequently, removing wax from their body will allow better penetration of the FPEs and cause death to the mealybugs. Similar findings have been reported by Khadem et al. (2022), who had proven the capability of Mexican mint FPE to remove the wax from *P. citri*.

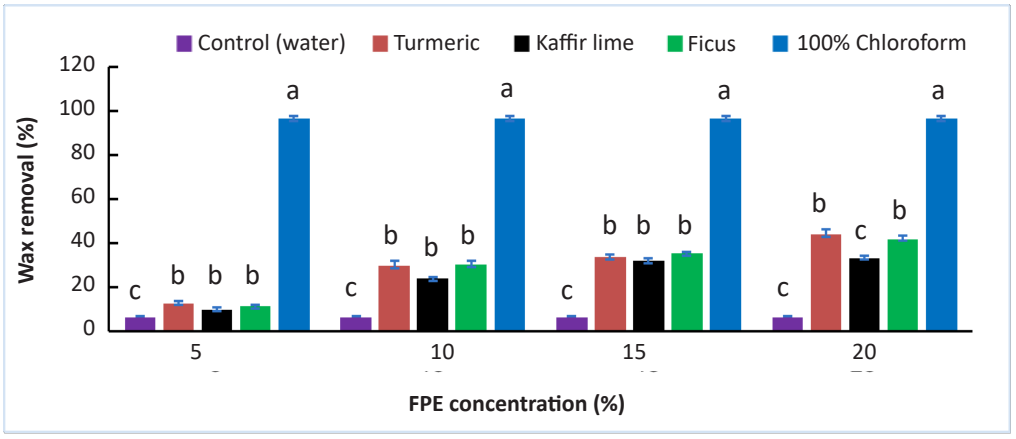


Figure 3. Percentage of *Phenacoccus solenopsis* wax removal (%) by different fermented plant extract (FPE) concentrations (%) at 72 hr post-treatment. Similar letters on bars corresponding to the similar response indicate no significant variation among the FPEs according to Tukey's test ($P < 0.001$). The vertical line on top of the bars corresponds to the standard error

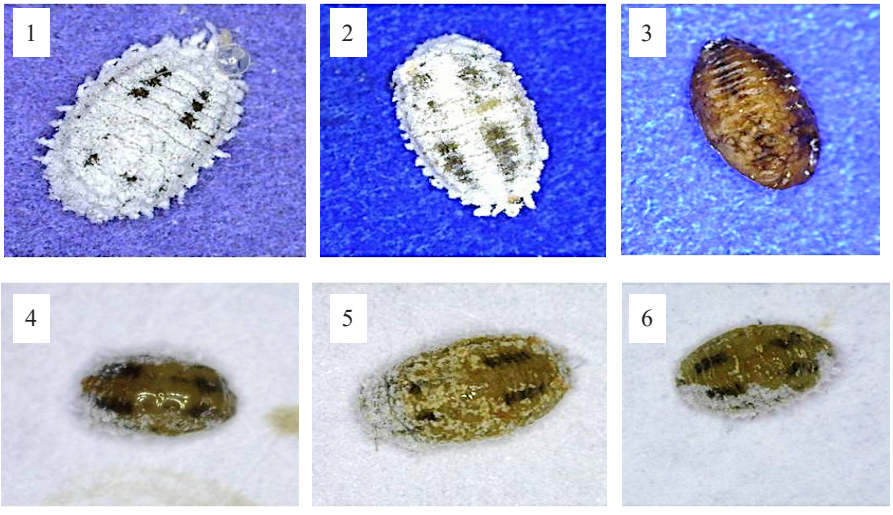


Figure 4. Effect of fermented plant extracts (FPEs) on the mealybug wax. 1) Untreated mealybug, 2) water-treated mealybug, 3) chloroform-treated mealybug, 4) turmeric FPE-treated mealybug, 5) ficus FPE-treated mealybug, and 6) kaffir lime FPE-treated mealybug

CONCLUSION

The present findings revealed the potentiality of FPEs in the control of *P. solenopsis*. There was variation in the mortality of *P. solenopsis* in response to different FPEs. The FPEs derived from ficus, kaffir lime and turmeric were effective in suppressing the *P. solenopsis* colonies on *H. rosa-sinensis* with an LC_{50} value of less than 20% (w/v). FPEs were also able to remove less than 50% of the mealybug wax, exposing the mealybugs to greater FPE penetration and resulting in the death of the mealybug. Selecting FPE with appropriate concentration for controlling *P. solenopsis* is crucial to avoid phytotoxic impact on plant growth. This study was conducted on a laboratory scale; thus, further studies may be required to evaluate the efficacy of FPEs under field conditions.

ACKNOWLEDGEMENTS

The authors thank the National Agricultural Technology Program-Phase II Project (NATP-2), BARC Component, Bangladesh Agricultural Research Council, for funding this project under grant number 6282508-10201 and Universiti Putra Malaysia (UPM) for providing research facilities during the study.

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