

## Potential Use of Fermented Plant Extracts as Biological Control Agents for Citrus Mealy Bugs, *Planococcus citri*

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### ABSTRACT

Management of mealy bugs, *Planococcus citri* is challenging due to its wide host range, presence of a waxy coating on the body, and high reproductive potentiality. The objective of the present study was to evaluate the insecticidal potential of different fermented plant extracts as potential biological control agents for citrus mealy bugs. The efficacy of the following fermented plant extracts on *P. citri* was estimated : onion (*Allium cepa*), garlic (*Allium sativum*), turmeric (*Curcuma longa*), aromatic ginger (*Kaempferia galanga*), lemongrass (*Cymbopogon citratus*), Mexican mint (*Plectranthus amboinicus*), variegated mintleaf (*Plectranthus madagascariensis*), peppermint (*Mentha × piperita*), kaffir lime (*Citrus hystrix*) and lime (*Citrus aurantiifolia*). More than 80% mealy bugs died after 120 h post-treatment with LC50 less than 10% (w/v) resulted by the fermented Mexican mint extract, fermented turmeric extract and fermented onion extract. The mealy bugs wax was detached after being treated with fermented plant extracts at concentrations above 10% (w/v). High phytotoxic (more than 40% leaf damage after 72 h post-treatment) effect was recorded in the fermented Mexican mint extract. These findings were/are considered as the first worldwide report on such interactions. The research findings reported in the present study revealed the potential use of fermented plant extract as biological control agent for *P. citri*, which will benefit mealy bugs control at a safer and environmentally-friendly approach.

**Key words :** Fermented plant extracts, mealy bugs, biological control, phytotoxicity, wax removal

### INTRODUCTION

Malaysia had about 198,435.03 ha of agricultural land cultivated with fruit crops, with an average production of 11.28 metric tonnes/ha in 2018 (DOA, 2018). Citrus crops occupied 2,836.32 ha of agricultural land with 25,421.59 metric tonnes of fruit production recorded in 2018 (DOA, 2018). Several insect-pests have been reported on the local citrus. *Planococcus citri* is a polyphagous pest which attacks many important cash crops, ornamental plants and fruit plants. They are soft-bodied, small plant-sucking insects that form the second largest scale insect family. The nymph and adult females have caused damage to many crops (Asiedu *et al.*, 2014). *P. citri* could transmit pathogens such as *Grape Vein Leaf Roll Virus* and *Grape Vein Virus* in grapes (Bertin *et al.*, 2016; Mruthunjayaswamy *et al.*, 2019). In various temperate and tropical

regions, the new shoots and leaves of plants in greenhouses and orchards are attacked by *P. citri*. They prefer to hide in sheltered areas and form thick colonies. The waxy coating of *P. citri* protects them from chemical contact (Asiedu *et al.*, 2014). Thus, control of *P. citri* has become challenging (Aldosary *et al.*, 2018). Fermented plant extracts (FPEs) are produced by fermentation of plant materials such as fruits peel or garden waste, and added with water, molasses, or brown sugar. They are multiuse solutions for household and farming (Neupane and Khadka, 2019). High concentration of acetic acid and low pH could be the key factors of the fermented enzyme to be effective in removing odor, dirt, stain, etc. The by-products such as acetic acid, "vinegar", alcohol and propionic acid can resolve insecticide resistance and provide alternative solution to the chemical management. Neupane and Khadka (2019) reported that FPEs

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could be used to control mosquitos, flies, rats, cockroaches, etc. Fermented kakawate and marigold extracts could reduce the number of whiteflies and 28-spotted beetles (Baloc *et al.*, 2015), while fermented *Acorus calamus*, *Coriandrum sativum* and *Zingiber officinale* and their combinations have shown good result in the larval mortality of *Spodoptera litura* (Subash and Raju, 2014). Daniels and Miller (2015) reported the successful use of acidic solutions such as acetic acid or vinegar to kill insects. Since acidic solutions have been proven to show positive control of mealy bugs in the field, the by-product of fermented plant waste could provide a cheaper source of acidic solution for mealy bug control. Therefore, this study was carried out to determine the efficacy and wax removing effect of fermented plant extracts against mealy bugs, *P. citri*. Phytotoxicity assay of fermented plant extracts was also tested on citrus leaves. Fermented plant extracts with insecticidal effects would be a green control measure for mealy bugs control as they are biodegradable, cheap and harmless to the users, consumers, non-target organisms and the environment.

## MATERIALS AND METHODS

*Planococcus citri* at different growth stages were collected from the naturally infested citrus plants around the campus of Universiti Putra Malaysia (UPM) from January 2019 till March 2020. The insects were brought to the Laboratory of Insect Pathology, UPM for rearing. The pumpkin (*Cucurbita pepo*) and sprouted potatoes (*Solanum tuberosum*) were washed with 0.5% (v/v) Clorox and then air-dried prior to mealy bugs feeding. After air-drying, the pumpkins and sprouted potatoes were kept in plastic containers (38 L × 28 H × 21 W cm). The insects were removed from the infested

leaves and released onto the pumpkins and sprouted potatoes with the help of a camel hairbrush. Insects were reared at 24–27°C±2°C and relative humidity ranging from 67 to 72±5% in the insectary according to the method described by Pawar *et al.* (2017).

The plant materials listed in Table 1 were used in this study. They were either purchased from the local markets or cultivated in the Faculty of Agriculture, University Putra Malaysia. Onion (*Allium cepa*), garlic (*Allium sativum*), turmeric (*Curcuma longa*), aromatic ginger (*Kaempferia galanga*), lemongrass (*Cymbopogon citratus*), Mexican mint (*Plectranthus amboinicus*), variegated mintleaf (*Plectranthus madagascariensis*), peppermint (*Mentha × piperita*), kaffir lime (*Citrus hystrix*) and lime (*Citrus aurantiifolia*) were used to control many insect-pests (Liu *et al.*, 2014; Sanei-Dehkordi *et al.*, 2016; Malar *et al.*, 2017; Mya *et al.*, 2017; Castillo-Sanchez *et al.*, 2018; Wanna and Kwang-Ngoen, 2019). They were proven to contain insecticidal compounds for insect control.

Fermented plant extracts (FPEs) were prepared according to the method of Prishanthini and Vinobaba (2014), Lanjar *et al.* (2015) and Naik and Naik (2015). The plant materials were washed with 0.1% (v/v) Clorox for 5 min, rinsed twice with distilled water, and then left to dry at room temperature. After drying, they were cut into 2–3 cm<sup>3</sup> in size with sterile scissors or knife on a sterile chop board. The plant materials were mixed with molasses and autoclaved distilled water at the ratio of 3:1:10 in clean plastic containers (4 L volume). Molasses was used to supply extra energy to the microorganisms. Three replications were made for each plant material. The gas as a by-product of the fermentation was released once in a week by opening the cap of each container in a laminar airflow. The containers were kept

**Table 1.** List of selected plant materials for fermentation

| S. No | Common name         | Scientific name                      | Family         | Source                | Plant parts |
|-------|---------------------|--------------------------------------|----------------|-----------------------|-------------|
| 1.    | Onion               | <i>Allium cepa</i>                   | Amaryllidaceae | Purchased from market | Bulb        |
| 2.    | Garlic              | <i>Allium sativum</i>                | Amaryllidaceae | Purchased from market | Bulb        |
| 3.    | Lemongrass          | <i>Cymbopogon citratus</i>           | Poaceae        | Cultivated in UPM     | Leaves      |
| 4.    | Mexican mint        | <i>Plectranthus amboinicus</i>       | Lamiaceae      | Cultivated in UPM     | Leaves      |
| 5.    | Peppermint          | <i>Mentha × piperita</i>             | Lamiaceae      | Purchased from market | Leaves      |
| 6.    | Variegated mintleaf | <i>Plectranthus madagascariensis</i> | Lamiaceae      | Cultivated in UPM     | Leaves      |
| 7.    | Aromatic ginger     | <i>Kaempferia galanga</i>            | Zingiberaceae  | Cultivated in UPM     | Leaves      |
| 8.    | Turmeric            | <i>Curcuma longa</i>                 | Zingiberaceae  | Purchased from market | Rhizome     |
| 9.    | Kaffir lime         | <i>Citrus hystrix</i>                | Rutaceae       | Purchased from market | Fruit       |
| 10.   | Lime                | <i>Citrus aurantiifolia</i>          | Rutaceae       | Purchased from market | Fruit       |

at room temperature for three months. After incubation, the FPEs were filtered through a 9 cm-diameter Whatman No. 1 filter paper placed on the Corning™ Falcon 50-ml conical centrifuge tubes. Filtrates were frozen overnight at -35°C prior to freeze-drying at -110°C.

The bioassay was conducted following the method of Majeed *et al.* (2018) with some modifications. Healthy citrus leaves were collected and brought back to the Laboratory of Insect Pathology, UPM. They were washed with 0.1% (v/v) Clorox for 5 min and then rinsed twice with distilled water. Freeze-dried fermented plant extracts were prepared in 5, 10, 15, 20 and 25% (w/v) as treatments, while the autoclaved distilled water was used as the negative control. Leaves with 9 cm in diameter were prepared and covered with wet cotton to prevent dehydration. Leaf discs were dipped in FPEs for 30 sec and then air-dried in laminar airflow. After drying, a single leaf disc was placed on a moist filter paper (9 cm in diameter) in a sterilized glass petri-dish (9 cm in diameter). Ten *P. citri* were released onto each treated leaf disc with the help of a camel-hair brush. The mealy bugs were checked for mortality under Dino-Eye eyepiece camera at 24, 48, 72, 96 and 120 h after treatment. The mealy bugs were considered dead if they did not move when touched with a camel-hair brush. The bioassay was carried out at 25±2°C and 67±10% relative humidity under complete randomized design (CRD) with 10 replications for each concentration. The percentage of mortality was calculated using the following formula :

$$\text{Mortality \%} = \frac{\text{Number of the dead insects}}{\text{Total number of test insects}} \times 100$$

The data were analyzed by using analysis of variance and the mean separation test using Tukey HSD at alpha 0.05. The effectiveness of FPEs in killing the mealy bugs was expressed in median lethal concentration (LC<sub>50</sub>) and analyzed by probit and logit analysis using POLO-Plus Version: 0.03 (LeOra Software, 2002-2020).

Chemical, disease and insect free citrus leaves were harvested and brought back to the Laboratory of Insect Pathology. They were washed with 0.1% (v/v) Clorox and dried at

room temperature. Leaves with 9 cm in diameter were selected and the petiole of each leaf was covered with wet cotton. The leaves were dipped in the FPEs at different concentrations (5, 10, 15, 20 and 25%). Leaves treated with distilled water served as negative control. Single treated citrus leaf was placed in a 9-cm diameter petri dish layered with a Whatman No. 1 filter paper at the bottom of the leaf. Five replications were conducted. The treated leaves were observed at 24 h interval until 72 h for leaf injury such as yellowing or lesion. The percentage of leaf injury was calculated using the following formula :

$$\% \text{ Leaf injury} = \frac{\text{Area of leaf injury}}{\text{Total area of leaf tested}} \times 100$$

The severity of leaf damage due to FPEs was rated according to the percentage of leaf injury (Table 2).

**Table 2.** Phytotoxic level of FPEs

| Rating | Leaf injury (%) | Severity    | Abbreviation |
|--------|-----------------|-------------|--------------|
| 0      | 0               | No          | N            |
| 1      | 1-10            | Low         | L            |
| 2      | 11-20           | Moderate    | M            |
| 3      | 21-30           | High        | H            |
| 4      | 31-40           | Very high   | VH           |
| 5      | 41-50           | Severe      | S            |
| 6      | >51             | Very severe | VS           |

FPEs were prepared in three concentrations, namely, 10, 15 and 20% (w/v). Chloroform in 100% (v/v) was served as positive control. Autoclaved distilled water was served as negative control. A total of 100 mealy bugs of similar size were weighted. Mealy bugs were placed in between two layers of 9 cm Whatman No. 1 filter papers and then soaked in either FPE, chloroform or autoclaved with distilled water for 30 and 60 sec, respectively (Fig. 1). The edge of the Whatman No. 1 filter papers was folded to avoid escape of mealy bugs during soaking. Both mealy bugs and Whatman No. 1 filter papers were air-dried on a piece of tissue paper placed in a tray under sterile condition. The mealy bugs were carefully removed from the Whatman No. 1 filter papers by using a camel-hair brush. The weight of the Whatman No. 1 filter papers was recorded before and after the treatment. This experiment was conducted with three replications.

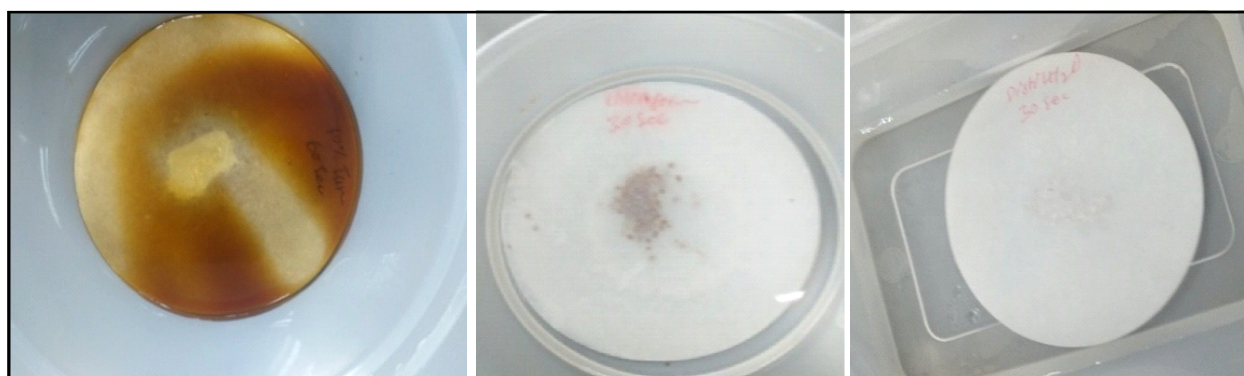


Fig. 1. Wax removing test : (a) Soaking of mealy bugs in FPE, (b) H<sub>2</sub>O and (c) Chloroform

## RESULTS AND DISCUSSION

The mortality rate of *P. citri* crawlers increased with the increase of concentration and exposure time of FPEs (Table 3). Majority of the FPEs had induced less than 50% crawler mortality after 24 h post-treatment except the fermented Mexican mint extract with more than 50% crawler mortality recorded for concentration of FPEs above 5% (w/v). The crawler mortality rate of fermented Mexican mint extract increased from 74-86% after 72 h post-treatment and then 83-100% after 120 h post-treatment. The fermented turmeric extract presented more than 40% crawler mortality at concentration 20% (w/v) and 25% (w/v) after 24 h post-treatment, however, these results were not significantly different from the fermented variegated mint, onion, lemongrass, peppermint, aromatic ginger, kafir lime and lime extracts. FPEs such as turmeric, onion and lemongrass showed

higher incidence of crawler mortality (more than 80% crawler mortality) at concentration higher than 10% (w/v) after 120 h post-treatment. The fermented garlic extract presented the least crawler mortality in all treatments as compared to other FPEs.

The fermented Mexican mint extract, fermented turmeric extract and fermented onion extract were highly toxic to *P. citri* with less than 2% (w/v) LC<sub>50</sub> value recorded after 120 h post-treatment (Table 4). The fermented lemongrass extract and fermented variegated extract required 4.65 and 6.14% (w/v) to cause 50% crawler mortality of *P. citri*, respectively. Other FPEs had recorded higher LC<sub>50</sub> value and the least toxic FPEs to *P. citri*.

The fermented Mexican mint extract, fermented turmeric extract and fermented onion extract were selected for phytotoxicity study based on their low LC<sub>50</sub> values. The phytotoxic effect of these FPEs was recorded after 24, 48 and 72 h post-treatment (Table 5).

**Table 3.** Mortality rates of *P. citri* after treated with FPEs

| Plant materials | Mortality of <i>P. citri</i> (%) |                  |                 |                  |                  |                  |                   |                   |                   |                   |                   |                   |                  |                   |                    |
|-----------------|----------------------------------|------------------|-----------------|------------------|------------------|------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------------------|-------------------|--------------------|
|                 | 24 h                             |                  |                 |                  |                  | 72 h             |                   |                   |                   |                   | 120 h             |                   |                  |                   |                    |
|                 | 5                                | 10               | 15              | 20               | 25               | 5                | 10                | 15                | 20                | 25                | 5                 | 10                | 15               | 20                | 25                 |
| Distilled water | 0 <sup>d</sup>                   | 0 <sup>d</sup>   | 0 <sup>c</sup>  | 0 <sup>d</sup>   | 0 <sup>d</sup>   | 0 <sup>f</sup>   | 0 <sup>g</sup>    | 0 <sup>g</sup>    | 0 <sup>f</sup>    | 0 <sup>e</sup>    | 0 <sup>e</sup>    | 0 <sup>i</sup>    | 0 <sup>f</sup>   | 0 <sup>f</sup>    | 0 <sup>f</sup>     |
| Variegated mint | 1 <sup>c</sup>                   | 13 <sup>cd</sup> | 17 <sup>c</sup> | 26 <sup>c</sup>  | 28 <sup>bc</sup> | 28 <sup>de</sup> | 37 <sup>de</sup>  | 54 <sup>bcd</sup> | 60 <sup>c</sup>   | 64 <sup>bcd</sup> | 46 <sup>bc</sup>  | 61 <sup>cde</sup> | 66 <sup>bc</sup> | 71 <sup>bc</sup>  | 80 <sup>bcd</sup>  |
| Onion           | 9 <sup>cd</sup>                  | 19 <sup>bc</sup> | 22 <sup>c</sup> | 26 <sup>c</sup>  | 30 <sup>bc</sup> | 51 <sup>bc</sup> | 57 <sup>bc</sup>  | 58 <sup>bc</sup>  | 65 <sup>abc</sup> | 75 <sup>abc</sup> | 72 <sup>a</sup>   | 75 <sup>abc</sup> | 80 <sup>ab</sup> | 85 <sup>ab</sup>  | 93 <sup>abc</sup>  |
| Turmeric        | 21 <sup>b</sup>                  | 29 <sup>b</sup>  | 40 <sup>b</sup> | 43 <sup>ab</sup> | 43 <sup>b</sup>  | 63 <sup>ab</sup> | 65 <sup>ab</sup>  | 71 <sup>ab</sup>  | 76 <sup>ab</sup>  | 79 <sup>ab</sup>  | 77 <sup>a</sup>   | 85 <sup>ab</sup>  | 88 <sup>a</sup>  | 92 <sup>a</sup>   | 94 <sup>ab</sup>   |
| Lemongrass      | 10 <sup>bcd</sup>                | 18 <sup>bc</sup> | 23 <sup>c</sup> | 29 <sup>bc</sup> | 32 <sup>bc</sup> | 34 <sup>cd</sup> | 52 <sup>bcd</sup> | 58 <sup>bc</sup>  | 64 <sup>abc</sup> | 68 <sup>abc</sup> | 51 <sup>b</sup>   | 69 <sup>bcd</sup> | 78 <sup>ab</sup> | 79 <sup>abc</sup> | 85 <sup>abcd</sup> |
| Peppermint      | 6 <sup>cd</sup>                  | 13 <sup>cd</sup> | 18 <sup>c</sup> | 23 <sup>c</sup>  | 2 <sup>bc</sup>  | 20 <sup>de</sup> | 42 <sup>cde</sup> | 44 <sup>cde</sup> | 54 <sup>cd</sup>  | 62 <sup>bcd</sup> | 29 <sup>cd</sup>  | 49 <sup>efg</sup> | 52 <sup>cd</sup> | 65 <sup>cd</sup>  | 72 <sup>de</sup>   |
| Mexican mint    | 41 <sup>a</sup>                  | 50 <sup>a</sup>  | 54 <sup>a</sup> | 55 <sup>a</sup>  | 63 <sup>a</sup>  | 74 <sup>a</sup>  | 78 <sup>a</sup>   | 80 <sup>a</sup>   | 80 <sup>a</sup>   | 86 <sup>a</sup>   | 83 <sup>a</sup>   | 88 <sup>a</sup>   | 91 <sup>a</sup>  | 95 <sup>a</sup>   | 100 <sup>a</sup>   |
| Aromatic ginger | 12 <sup>bc</sup>                 | 18 <sup>bc</sup> | 21 <sup>c</sup> | 31 <sup>bc</sup> | 35 <sup>bc</sup> | 24 <sup>de</sup> | 48 <sup>cd</sup>  | 50 <sup>cde</sup> | 58 <sup>c</sup>   | 59 <sup>cd</sup>  | 29 <sup>cd</sup>  | 52 <sup>def</sup> | 55 <sup>cd</sup> | 66 <sup>cd</sup>  | 70 <sup>de</sup>   |
| Garlic          | 3 <sup>cd</sup>                  | 8 <sup>cd</sup>  | 15 <sup>c</sup> | 19 <sup>c</sup>  | 23 <sup>c</sup>  | 14 <sup>ef</sup> | 16 <sup>fg</sup>  | 17 <sup>fg</sup>  | 30 <sup>d</sup>   | 46 <sup>d</sup>   | 16 <sup>de</sup>  | 25 <sup>h</sup>   | 27 <sup>e</sup>  | 38 <sup>e</sup>   | 68 <sup>e</sup>    |
| Kafir lime      | 7 <sup>cd</sup>                  | 14 <sup>c</sup>  | 21 <sup>c</sup> | 28 <sup>c</sup>  | 30 <sup>bc</sup> | 24 <sup>de</sup> | 29 <sup>ef</sup>  | 37 <sup>de</sup>  | 54 <sup>c</sup>   | 57 <sup>cd</sup>  | 33 <sup>bcd</sup> | 34 <sup>gh</sup>  | 51 <sup>cd</sup> | 64 <sup>cd</sup>  | 77 <sup>cde</sup>  |
| Lime            | 6 <sup>cd</sup>                  | 11 <sup>cd</sup> | 16 <sup>c</sup> | 23 <sup>c</sup>  | 29 <sup>bc</sup> | 27 <sup>de</sup> | 30 <sup>ef</sup>  | 33 <sup>ef</sup>  | 37 <sup>d</sup>   | 69 <sup>abc</sup> | 35 <sup>bc</sup>  | 39 <sup>fgh</sup> | 47 <sup>d</sup>  | 49 <sup>de</sup>  | 79 <sup>bcd</sup>  |

Means within columns with different superscripts are significantly different ( $P \leq 0.05$ ).

**Table 4.** Toxicity of FPEs on *P. citri* after 120 h post-treatment

| Treatment       | LC <sub>50</sub> | 95% CL         | Slope±S. E. | Heterogeneity | df. |
|-----------------|------------------|----------------|-------------|---------------|-----|
| Mexican mint    | 1.26             | 0.22 to 2.52   | 1.44+-0.33  | 0.71          | 48  |
| Turmeric        | 1.38             | 0.16 to 2.89   | 1.22+-0.298 | 1.13          | 48  |
| Onion           | 1.67             | 0.08 to 3.68   | 1.02±0.26   | 1.37          | 48  |
| Lemongrass      | 4.65             | 2.06 to 6.68   | 1.42±0.24   | 1.60          | 48  |
| Variegated mint | 6.14             | 3.17 to 8.36   | 1.18±0.24   | 1.21          | 48  |
| Aromatic ginger | 10.95            | 8.86 to 13.03  | 1.50±0.24   | 0.51          | 48  |
| Peppermint      | 11.45            | 9.42 to 13.55  | 1.55±0.24   | 0.70          | 48  |
| Kaffir lime     | 12.14            | 10.24 to 14.22 | 1.67±0.24   | 0.62          | 48  |
| Lime            | 13.17            | 10.67 to 16.29 | 1.30±0.24   | 0.60          | 48  |
| Garlic          | 22.54            | 19.03 to 28.93 | 1.82±0.26   | 0.91          | 48  |
| Distilled water | 0                | 0              | 0           | 0             | 48  |

Concentration of FPEs at 5 and 10% (w/v) did not induce phytotoxicity to citrus leaves after 24, 48 and 72 h post-treatment. When the concentration of FPE and the post-treatment time increased, different levels of phytotoxic effect were observed. Among the tested FPEs, fermented Mexican extract had induced higher phytotoxic effect to citrus leaves compared to the fermented turmeric extract and fermented onion extract at different time intervals. The severity level of the phytotoxic effect caused by the fermented Mexican mint extract increased with time and concentrations, and it became severe after 72 h post-treatment. Citrus leaves treated with the fermented Mexican mint extract exhibited severe chlorosis symptom after 72 h post-treatment. The fermented turmeric extract and fermented onion extract also showed similar trend of phototoxic effect to citrus leaves, however, their phototoxic level was moderate compared to the fermented Mexican mint.

**Table 5.** Phytotoxic effect of FPEs on citrus leaves

| Factor                   | Phytotoxic percentage (level) |                         |                         |
|--------------------------|-------------------------------|-------------------------|-------------------------|
|                          | 24 h                          | 48 h                    | 72 h                    |
| <b>Treatment</b>         |                               |                         |                         |
| Mexican mint             | 12.48 <sup>a</sup> (M)        | 25.28 <sup>a</sup> (H)  | 41.38 <sup>a</sup> (S)  |
| Turmeric                 | 1.56 <sup>b</sup> (L)         | 10.88 <sup>b</sup> (M)  | 17.12 <sup>b</sup> (M)  |
| Onion                    | 3.68 <sup>c</sup> (L)         | 8.96 <sup>c</sup> (L)   | 15.6 <sup>c</sup> (M)   |
| Distilled water          | 0.00 <sup>d</sup> (N)         | 0.00 <sup>d</sup> (N)   | 0.00 <sup>d</sup> (N)   |
| <b>Concentration (%)</b> |                               |                         |                         |
| 5                        | 0.00 <sup>d</sup> (N)         | 0.00 <sup>d</sup> (N)   | 0.00 <sup>d</sup> (N)   |
| 10                       | 0.00 <sup>d</sup> (N)         | 0.00 <sup>d</sup> (N)   | 0.00 <sup>d</sup> (N)   |
| 15                       | 1.85 <sup>c</sup> (L)         | 6.8 <sup>c</sup> (L)    | 16.85 <sup>c</sup> (M)  |
| 20                       | 5.25 <sup>b</sup> (L)         | 17.83 <sup>b</sup> (M)  | 33.50 <sup>b</sup> (VH) |
| 25                       | 15.05 <sup>a</sup> (M)        | 32.15 <sup>a</sup> (VH) | 42.28 <sup>a</sup> (S)  |

Means within columns with different superscripts are significantly different (P≤0.05).

The fermented Mexican mint extract, fermented turmeric extract and fermented onion extract were further tested on *P. citri*. With increasing concentration and time of FPE treatment, the yield of wax also increased (Table 6). The fermented onion extract showed the highest mealy bug wax yield compared to fermented Mexican mint extract, fermented turmeric extract and the chloroform after 30 and 60 sec post-treatment. The fermented turmeric extract performed better than chloroform after 30 sec post-treatment, but the wax yield dropped after 60 sec post-treatment. The fermented Mexican mint extract had the least effect on the mealy bugs wax among the tested FPEs in this study.

**Table 6.** Yield of mealy bugs wax after treated with FPEs

| Treatment       | Yield of wax (mg) |                   |                   |                   |                   |                   |
|-----------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
|                 | 30 sec            |                   |                   | 60 sec            |                   |                   |
|                 | 10%               | 15%               | 20%               | 10%               | 15%               | 20%               |
| Turmeric        | 5 <sup>ab</sup>   | 7 <sup>b</sup>    | 9.4 <sup>b</sup>  | 7.2 <sup>b</sup>  | 8.9 <sup>c</sup>  | 11.3 <sup>b</sup> |
| Mexican mint    | 4.1 <sup>b</sup>  | 5.6 <sup>c</sup>  | 7.7 <sup>c</sup>  | 6.6 <sup>b</sup>  | 7.9 <sup>c</sup>  | 9.8 <sup>b</sup>  |
| Onion           | 6.8 <sup>a</sup>  | 10.7 <sup>a</sup> | 13.1 <sup>a</sup> | 7.6 <sup>b</sup>  | 15 <sup>a</sup>   | 19 <sup>a</sup>   |
| Chloroform      | 6 <sup>ab</sup>   | 6.1 <sup>bc</sup> | 6.2 <sup>d</sup>  | 11.2 <sup>a</sup> | 11.9 <sup>b</sup> | 12.7 <sup>b</sup> |
| Distilled water | 2 <sup>c</sup>    | 2.1 <sup>d</sup>  | 2.1 <sup>e</sup>  | 3.2 <sup>c</sup>  | 3.8 <sup>d</sup>  | 4.4 <sup>c</sup>  |

Means within columns with different superscripts are significantly different (P≤0.05).

Fermented plant extracts (FPEs) have been evaluated for their potential use in insect-pest control. In the present study, the FPEs derived from onion (*Allium cepa*), garlic (*Allium sativum*), turmeric (*Curcuma longa*), aromatic ginger (*Kaempferia galanga*), lemongrass (*Cymbopogon citratus*), Mexican mint (*Plectranthus amboinicus*), variegated mintleaf (*Plectranthus madagascariensis*), peppermint (*Mentha × piperita*), kaffir lime (*Citrus hystrix*) and lime (*Citrus aurantiifolia*) showed different degree of

positive insecticidal efficacy against *P. citri*. This will be the first record on positive response of FPEs against *P. citri*. Among the FPEs tested, the fermented Mexican mint extract, fermented turmeric extract and fermented onion extract showed promising efficacy to *P. citri* as more than 80% mealy bugs died after 120 h post-treatment. Their  $LC_{50}$  value was also recorded less than 2% (w/v) after 120 h post-treatment. The least effective FPE was the fermented garlic extract. Different types of plant material produced different types of secondary metabolites during fermentation (Omarini *et al.*, 2020). Some of these secondary metabolites acted as insecticides to insect such as *Callosobruchus maculatus*, *Bemisia tabaci* and *Myzus persicae* (Olukunle *et al.*, 2018).

Among the FPEs tested in the present study, the fermented Mexican mint extract showed the highest toxicity to *P. citri*. Mentha family (mint) contained natural compounds such as ketone monoterpenes which induced mortality to insects (Sánchez-Borzone *et al.*, 2017). Dogan and Tornuk (2019) reported that fermentation protected the bioactive compounds and antioxidants of mint from degradation and increased their bioavailability. Three different types of fermented mint leaves were tested against *P. citri* in the present study, and the best result was obtained from the fermented Mexican mint extract. This could be due to a higher level of bioactive compounds in the fermented Mexican mint extract compared to those of fermented variegated mint extract and fermented peppermint extract.

Fermented turmeric extract and fermented onion extract were also proven toxic to *P. citri* and caused 50% crawler mortality with concentration less than 2% (w/v) in the present study. Turmeric is a member of Zingiberaceae family and widely used as a functional food ingredient. It contained natural compound such as ar-turmerone which induced mortality to insects and weeds (Ali *et al.*, 2014; Kumar *et al.*, 2017; Ibáñez and Blázquez, 2019). Fermentation of turmeric proved an increase of the curcumin content which was a bioactive compound with anti-inflammatory activity (Yong *et al.*, 2019). It is believed that other bioactive compounds such as ar-turmerone might have higher yield generated during fermentation. Onion had

been used as a pest control intercrop in organic farming and repellent for insects (Yirankinyuki *et al.*, 2018). It has reported good insecticidal activity in the form of essential oil against insects and weeds (Kumar *et al.*, 2017; Ibáñez and Blázquez, 2019), however, information on the fermented onion as biopesticide is lacking. Other FPEs such as fermented lemongrass, aromatic ginger, peppermint, kaffir lime, lime and garlic extracts had exhibited moderate to low insecticidal activity to *P. citri*. Their mortality rate was lower than those reported in the form of essential oil (Pumnuan and Insung, 2016). Mixture of different FPEs in the present study could be a good alternative to enhance the efficacy of FPEs against mealy bugs.

Application of chemical, botanical, or biological pesticides may induce certain degree of leaf injury. Thus, phytotoxicity assessment is required for registration of plant protection products (Ambarish *et al.*, 2017). In the present study, the fermented Mexican mint extract induced severe leaf injury, while the fermented turmeric extract and onion extract had caused moderate leaf injury after 72 h post-treatment at higher concentration of FPE [ $> 20\%$  (w/v)]. The phytotoxic level increased with time and concentration of the FPEs. In view of the severe leaf injury caused by fermented Mexican mint extract in the present study, fermented turmeric extract and fermented onion extract will be recommended to be used as biocontrol agent of *P. citri* in the field.

The mealy bugs wax was detached from the mealy bugs body after treated with FPEs at concentration above 5%. The wax yield increased with concentration and time in all FPEs except the fermented turmeric extract. The fermented onion extract showed higher degree of wax removal than chloroform, which was a chemical commonly used in dissolving wax (Divya and Kalyanasundaram, 2019). The fermented Mexican mint extract had similar wax removing efficiency as chloroform at different time exposure, while the fermented turmeric extract did not improve in the wax removal efficiency with time exposure. Wax is a protective layer of mealy bugs. The reduction of wax on their body will allow better penetration of chemical, botanical, or biological pesticide to increase their functional effect on the target insects.

The present study demonstrated the possibility of FPEs in controlling mealy bugs, *P. citri*. The data will serve as fundamental approach to explore more potential FPEs for the control of mealy bugs. The selection of FPE for pest control is crucial to ensure effective control of mealy bugs. Fermented Mexican mint extract, fermented turmeric extract and fermented onion extract displayed a remarkable control on *P. citri*. However, due to high phytotoxicity, effect of fermented Mexican mint extract, fermented onion extract and fermented turmeric extract is recommended for further study on the effective application mode in the field. It is also important to explore the chemical composition of FPEs because they may contain more comprehensive information for the selection of effective FPEs in insect-pest control.

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