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Repellent Ability of Encapsulated Guava Leaves Extract and Horticultural Mineral Oil (HMO) on the Feeding Behaviour of *Diaphorina citri*

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ABSTRACT

Repellency is one of strategies to reduce *Diaphorina citri* attack. Guava leaf extract (GLE) and horticultural mineral oil (HMO) are well-known as repellents against *D. citri* but have short persistence. This research was conducted to increase the persistence of GLE and HMO extracts by using simultaneous co-delivery as part of environmentally friendly control of *D. citri*. The treatments used were 5% GLE, 5% HMO, 5% GLE + 5% HMO, 5% encapsulated GLE, 5% encapsulated HMO, 5% encapsulated GLE + 5% encapsulated HMO, and aquadest as control. The result shows that 5% HMO, 5% GLE + 5% HMO, and 5% encapsulated GLE have a repellency effect on *D. citri*. In the non-encapsulated treatments, the percentage of *D. citri* stayed at treated citrus leaves increased significantly at 9 and 12 hours after application except 5% GLE + 5% HMO. Exposure to 5% GLE, 5% HMO, and 5% encapsulated GLE. The most dominant of GLE compounds was lactose and d-Glycero-l-gluco-heptose. The most dominant of HMO compounds were 17-Pentatriacontene, Tetrapentacontane 1,54-dibromo- and tert-Hexadecanethiol. Encapsulation of 5% GLE can increase the persistence of GLE as a repellent compound for *D. citri*, but this effect does not occur in HMOs.

1. Introduction

Citrus production in both Indonesia and globally faces significant challenges due to Citrus Vein Phloem Degeneration (CVPD), also known as Huanglongbing (HLB) (Nurhadi 2015; Gómez-Flores *et al.* 2019). The causative agents of CVPD are *Candidatus Liberibacter asiaticus* and *Candidatus Liberibacter americanus*, which are associated with the Asian and American forms of the disease, respectively (Teixeira *et al.* 2005). In Asia, the disease is transmitted by the Asian citrus psyllid (*Diaphorina citri*) (Bové 2006; Monzo and Stansly 2017). Both adult psyllids and 4th-5th instar nymphs can transmit CVPD throughout their lifespan (Hung *et al.* 2004).

Effective management of CVPD includes the use of CVPD-free planting materials, removal of infected trees, and large-scale vector control (Zhou 2020). Currently, managing *D. citri* primarily depends on synthetic insecticides (Monzo and Stansly 2017; Alquézar *et al.* 2021). However, these chemicals often show limited efficacy and pose environmental concerns. As an alternative, plant-based insecticides derived from sources such as tobacco shoots, chrysanthemum flowers, bitters, orange peels (Wuryantini *et al.* 2020, 2021), and neem seeds (Santos *et al.* 2015) have demonstrated efficacy in laboratory settings. Additional plant-derived substances, including lavender, citronella (Kongkaew *et al.* 2011; Geetha and Roy 2014), rapeseed oil, and quassia (European Food Safety Authority 2014; Dalimunthe and Rachmawan 2017), exhibit repellent properties. Other natural insecticides include pyrethrum, neem, tuba root, and essential oils from cashew, betel leaves, cloves, and lemongrass (Braswell *et al.* 2020).

Diaphorina citri utilizes particular volatile compounds as chemical signals to detect its host

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plants and to select plant parts that are not occupied by other individuals (Silva *et al.* 2023).Similarly, natural enemies of pests use these volatile compounds to locate their prey (Kolanthasamy *et al.* 2023). Previous studies have highlighted the insecticidal potential of young guava leaves, with their volatile compounds exhibiting a repellent effect against *D. citri*, although their effectiveness diminishes over time (Poerwanto & Solichah 2020, 2021).

Research on guava leaves extract (GLE) has demonstrated its effectiveness as a repellent for adult *D. citri* (Poerwanto 2023). Phytochemical analysis of GLE identified secondary metabolites such as steroids, flavonoids, phenol hydroquinones, saponins, and tannins (Satiyarti *et al.* 2019). Horticultural mineral oil (HMO) has also shown repellent effects against *D. citri* (Tofangsazia *et al.* 2018). However, its high cost and limited availability in Indonesia pose challenges. Despite these limitations, HMO remains a preferred option for pest management due to its non-toxicity to plants and animals, ease of use, low risk, and cost-effectiveness (Nile *et al.* 2019). Application of HMO during the flush period has been shown to reduce *D. citri* populations by 56.7% to 61.3% (Poerwanto 2010), likely by masking host plant volatiles or releasing repellent compounds (Poerwanto *et al.* 2012). Additionally, HMO-treated plants have been observed to attract parasitoids (Poerwanto & Brotodjojo 2011) and predators (Poerwanto 2010).

In an interesting observation, the presence of one guava plant among eight citrus trees was enough to prevent *D. citri* infestations and CVPD attacks (Pustika *et al.* 2007). Volatiles specific to GLE were found to repel *D. citri* (Poerwanto & Solichah 2019), with the repellent effect varying depending on the guava variety and leaves age (Poerwanto & Solichah 2020). The repellant effect of GLE was higher on young guava leaves than on medium and old ones (Poerwanto *et al*. 2024). This suggests potential for GLE as a natural control method against *D. citri*. Guava leaves produce a variety of volatile compounds, including sesquiterpenes (Sagrero-Nieves *et al.* 1994; George *et al.* 2016), and some of these volatiles, such as green leaf volatiles, are known to repel insects (Soares *et al.* 2007). The effectiveness of these compounds is concentration-dependent (Zaka *et al.* 2011), and dimethyl disulfide, produced when guava leaves are injured, also has a repellent effect on *D. citri* (Onagbola *et al.* 2011).

Interestingly, guava plant volatiles are not expected to repel *D. citri's* natural predators, which are essential for biological control. When plants are attacked by pests, they release herbivore-induced plant volatiles (HIPVs), which serve as signals for predators. The quantity and type of HIPVs vary depending on the pest species and population (Backer *et al.* 2015). HIPVs produced in response to specific pest attacks are highly specialized and remain effective in attracting natural enemies, even against different pest species (Tan & Liu 2014). More resistant plants tend to produce lower levels of volatile methyl salicylate compared to more susceptible plants, which in turn attract more predators (Kersch-Becker *et al.* 2017).

One of the challenges in utilizing repellent compounds is their instability during storage and transportation, necessitating the development of technologies to improve the stability of these formulations. Enhancing the effectiveness of repellent compounds can be achieved by processing them into nanoparticles, which allow for simultaneous co-delivery (do Nascimento Junior *et al.* 2021). However, this topic has not been extensively studied, particularly in the context of co-delivering plant extracts and mineral oils using encapsulation nanotechnology. The specific aim of this research is to enhance the persistence of guava leaves extract (GLE) and horticultural mineral oil (HMO) through simultaneous co-delivery, contributing to an environmentally friendly approach to control *D. citri*.

2. Materials and Methods

2.1. Insect Rearing

This study was an experimental research conducted in a greenhouse. Rearing of *Diaphorina citri* was carried out under controlled conditions. Disease-free adult *Diaphorina citri* used in this study was obtained in May 2023 from the Research Centre for Citrus and Subtropical Fruits (BALITJESTRO), Malang, Indonesia. Fifty orange jasmine (*Murraya paniculata*) plants served as host plants. These were grown in plastic pots (30 cm diameter, 20 cm height) and placed inside gauze cages measuring 60 × 60 × 100 cm. Newly emerged buds following pruning were utilized as oviposition sites. Greenhouse conditions were maintained at a temperature of 26–30°C and relative humidity of 60–70%.

2.2. Guava Leaves Extraction

The apical shoots (first and second leaves from the tip) of guava (*Psidium guajava*) were oven-dried at 40°C for seven days. Once dried, the leaves were ground using an electric grinder and subsequently sieved to obtain fine powder. The resulting leaves powder was stored in airtight containers. The leaves powder was processed by a maceration method with methanol overnight. The supernatant that was obtained from filtered extract with a filter paper then was evaporated in a rotary evaporator at a temperature of 40°C up to the minimum volume.

2.3. Nanoparticles Encapsulation of GLE and HMO

Encapsulation was carried out using chitosan by ionic gelation method based on Maluin *et al.* 2019. The HMO nC21 Sunspray Ultra Fine® (Amtrade Pty Ltd, Melbourne, Victoria, Australia) was also treated as well as GLE. In brief, a chitosan solution was prepared by dissolving 5 mg/mL of chitosan in 100 mL acetic acid (1.0% v/v). Separately, GLE and HMO were each dissolved at a concentration of 10 mg/mL in 100 mL of N,N-dimethylformamide. These solutions were then gradually mixed into the chitosan solution under continuous stirring to achieve homogeneity. Subsequently, 2% (v/v) Tween-80 was added as a stabilizing agent to prevent particle aggregation. A concentration of 40 mL of sodium tripolyphosphate (TPP) with concentrations of 20 mg/mL was prepared in deionized water separately. A sodium TPP solution was slowly introduced into the chitosan mixture drop by drop using a burette, with continuous stirring to ensure uniform mixing. The process was carried out until a final TPP-to-chitosan volume ratio of 1:2.5 was reached. The resulting dispersion was subjected to centrifugation at 40,000 rpm for 10 minutes to separate the nanoparticles. After discarding the supernatant, the collected chitosan-GLE and HMO nanoparticle pellets were freeze-dried overnight to prepare them for subsequent analyses.

2.4. Effect on *D. citri* Host Finding Behaviour

Repellent ability of citrus twigs with two leaves dipped with 5% GLE, 5% HMO, 5% GLE + 5% HMO, 5% encapsulated GLE, 5% encapsulated HMO, 5% encapsulated GLE + 5% encapsulated HMO in five seconds, and undipped leaves as control was determined by conducting non-choice tests. The twigs were plugged in floral foam in an open plastic cup. Ten psyllids were starved for 30 minutes and placed in another plastic cup. The aroma source cup and insect cup were placed in a circular plastic cage Ø 30 cm, height 20 cm. The number of psyllids stayed in the aroma source cup, stayed in the insect cup, and moved to the cage except the aroma source cup and insect cup were recorded (Poerwanto and Solichah 2021). Exposure to psyllids was carried out from 3 until 12 hours. All treatments were replicated 6 times. The data obtained were statistically analyzed using analysis of variance (ANOVA) to determine significant differences among treatments.

2.5. Effect on Leaves Growth

The effect was observed on citrus buds and on citrus shoots. Effect on leaves growth was carried out by applying the treatment to the shoots with two open leaves of citrus plants. Pruning was conducted to produce buds. The effect on buds growth was carried out by applying the treatment to newly emerged buds of citrus plants. The treatments used were 5% GLE, 5% HMO, 5% encapsulated GLE, and aquadest as control. Each treatment was replicated six times. Observations on bud length, leaf length, leaf count, and signs of phytotoxicity were conducted weekly over a four-week period following the day of application. The data obtained were statistically analyzed using analysis of variance (ANOVA) to determine significant differences among treatments.

2.6. Evaluation by Gas Chromatography-Mass Spectrometry

GLE and HMO compounds were characterized through GC-MS analysis. GC-MS shows the list of phytochemical compounds based on retention time and molecular weight of each compound presented in the chromatogram. GC-MS was carried out at Integrated Laboratory for Research and Testing, Universitas Gadjah Mada.

3. Results

3.1. Effect on *D. citri* Host Finding Behaviour

Table 1 shows that 5% HMO, 5% GLE + 5% HMO, and 5% encapsulated GLE have a repellency effect to *D. citri.* They were significantly different in control at 3, 6, 9, and 12 hours after application. The ability of 5% GLE decreased and was not significantly different from control at 6, 9, and 12 hours after application. Encapsulation process to 5% GLE increases the repellency at 6, 9, and 12 hours after application. On the other hand, encapsulation to 5% HMO and 5% GLE + 5% HMO decreases the repellency at 3, 6, 9, and 12 hours after application. 5% encapsulated HMO had no significant effect (Table 1), however HMO without encapsulation has a high level of effectiveness, but after the encapsulation process (5% encapsulated HMO) has low effectiveness. *D. citri* tends to choose 5% encapsulated HMO over 5%

Table 1. The mean (± SE) percentage of *Diaphorina citri* stayed at citrus leaves treated by 5% GLE, 5% HMO, a combination of 5% GLE and 5% HMO, 5% encapsulated GLE, 5% encapsulated HMO, encapsulated combination of 5% GLE and 5% HMO

Treatments	Percentage number of *Diaphorina citri* entered into treatment (%)			
	3rd hour	6th hour	9th hour	12th hour
Control	50.00±9.13[a]	47.50±8.54[ab]	52.50±10.31[a]	55.00±9.57[a]
5% GLE	10.00±4.08[bc]	27.50±8.54[bcd]	35.00±5.00[ab]	42.50±4.79[ab]
5% HMO	5.00±2.89[c]	2.50±2.50[d]	10.00±4.08[c]	20.00±7.07[bc]
5% GLE + 5% HMO	10.00±4.08[bc]	17.50±6.29[cd]	20.00±4.08[bc]	17.50±7.50[c]
5% Encapsulated GLE	12.50±7.50[bc]	12.50±9.46[d]	20.00±8.16[bc]	27.50±10.31[bc]
5% Encapsulated HMO	45.00±10.41[a]	62.50±8.54[a]	57.50±7.50[a]	57.50±7.50[a]
5% Encapsulated GLE + 5% Encapsulated HMO	30.00±10.00[ab]	40.00±12.25[abc]	40.00±9.13[ab]	37.50±4.79[abc]

Mean (± SE) followed with the same letters are not significantly different in the column ($P<0.05$)

HMO without encapsulation. Meanwhile, 5% GLE and 5% encapsulated GLE had similar effectiveness at 3 hours. However, as exposure hours increased, 5% encapsulated GLE was more effective than 5% GLE.

Figure 1 shows that in the non-encapsulated treatments, the percentage of *D. citri* stayed at treated citrus leaves increased significantly at 9 and 12 hours after application except 5% GLE + 5% HMO. However, encapsulation treatments had kept the number from increasing significantly. The repellency effect is retained for a long time with encapsulation. The percentage of *D. citri* stayed at 5% GLE increase at 9 hours after application. The percentage of *D. citri* stayed at 5% HMO increase 12 hours after application. At the encapsulation of 5% GLE, 5% HMO, and 5% GLE + 5% HMO, the percentage of *D. citri* stayed did not increase.

3.2. Effect on Leaves Growth

The effect of 5% GLE, 5% HMO, 5% encapsulated GLE, and control treatments to leave and buds growth were shown in Figure 2 respectively. The leaves growth parameters were the increasing of leaves number, leaves length and buds length (mm). The effect of 5% GLE, 5% HMO, 5% encapsulated GLE, and control treatments to leave and buds growth were not significantly different (Table 2). It shows that exposure to5% GLE, 5% HMO, and 5% encapsulated GLE did not have a bad effect on bud and leaf growth. The increase on the 5% encapsulated GLE is tending mostly highest on leaves number, leaves length and buds length than other treatments. The effect of 5% GLE, 5% HMO and 5% encapsulated GLE on growth performance were not significantly different.

3.3. Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

The results showed that there were 137 peaks and 52 possible compounds found on HMO, meanwhile 70 peaks and 75 found on GLE with differences of Similarity Index (SI) on Figure 3. The most dominant of GLE compounds was carried out on the 44th peak with the retention area was 29.72%. They are quinic acid, lactose, and d-Glycero-l-gluco-heptose. While the most dominant of HMO compounds was carried out on 76th peaks with the retention area was 6.44%. Its compounds were 17-Pentatriacontene, Tetrapentacontane 1,54-dibromo- and tert-Hexadecanethiol Table 3.

4. Discussion

Evidence supporting the role of guava leaves in reducing *D. citri* infestations has been well documented. Studies show that citrus intercropped with guava significantly reduces *D. citri* populations, and citrus nurseries interplanted with guava also experience a decline in the incidence of CVPD or HLB (Gottwald *et al.* 2014). Olfactometer tests have demonstrated that volatile compounds from guava leaf extract (GLE) reject the presence of *D. citri*, with the repellent effect varying across the varieties of guava and decreasing as the leaves age (Poerwanto and Solichah 2020). Interestingly, GLE volatiles attract generalist predators such as Menochilus sexmaculatus adults, showing a stronger attraction effect on guava leaves compared to citrus leaves, thereby offering a dual suppression strategy for *D. citri* populations (Poerwanto and Solichah 2022). To enhance the persistence of the

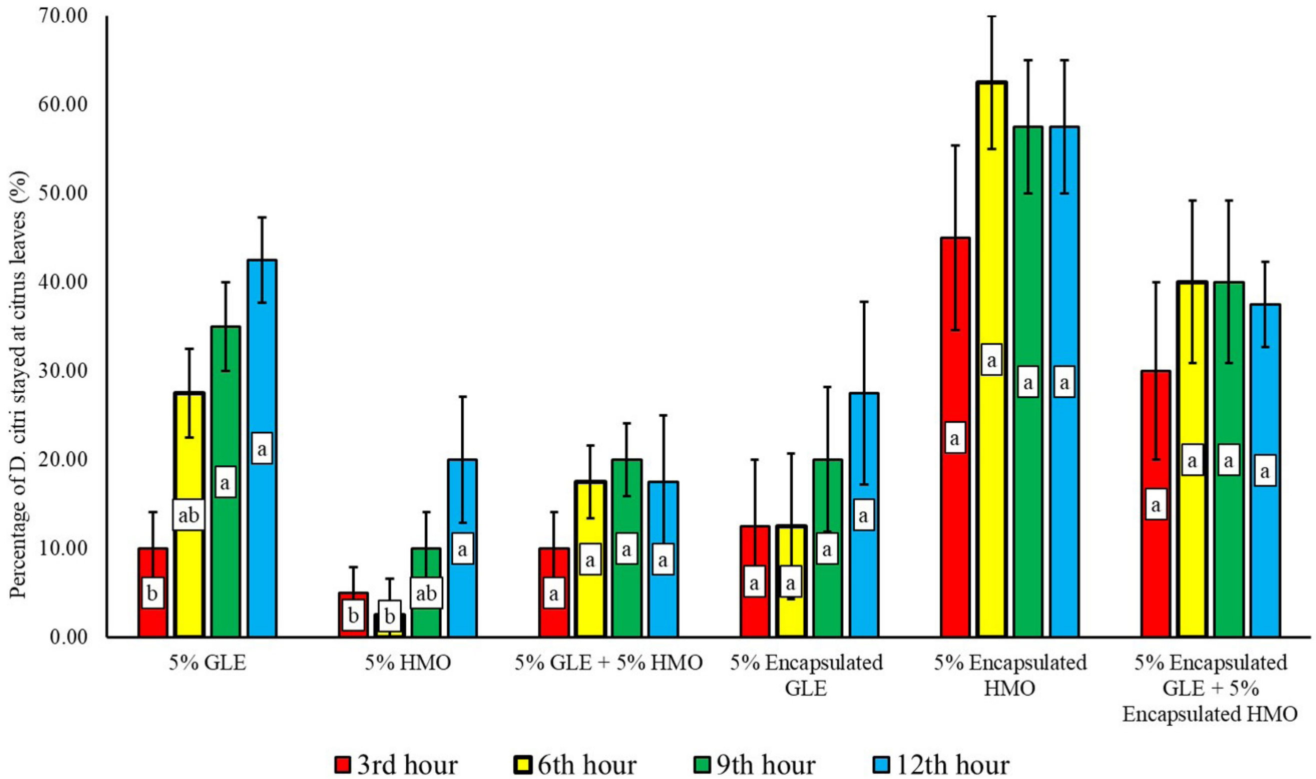

Figure 1. The mean (± SE) percentage of *D. citri* stayed at citrus leaves based on periods of observations of 3rd, 6th, 9th, 12th hours treated by 5% GLE, 5% HMO, 5% GLE + 5% HMO, 5% encapsulated GLE, 5% encapsulated HMO, 5% encapsulated GLE + 5% encapsulated HMO. Bars with the same letter are not significantly different within the treatment (P<0.05)

repellent effect, encapsulation technology can be applied. This technique involves the creation of microcapsules with active ingredients at the core, encased in chitosan, which facilitates the slow release of the repellent (Raza *et al.* 2020).

Research indicates that encapsulation techniques in pest control can enhance effectiveness. Encapsulated formulations of rosemary and Zataria essential oils enable the controlled release of pesticides, optimizing their biological activity over extended periods. In contrast, unformulated oils exhibit less than 10% insect mortality after 25 days, with their effectiveness declining within 72 hours, whereas encapsulated versions demonstrate sustained impact (Ahsaei *et al.* 2020). The encapsulation efficiency of citronella essential oil (CEO) for controlling Spodoptera littoralis (Boisd.) reached 61.8±1% in chitosan nanoparticles (CSNPs) and 90.8±1% in cellulose nanofiber (CNF) systems, achieving prolonged persistence and higher larval mortality. After two weeks, CEO release from CSNP and CSNPs/CNF nanosystems was 100% and %, respectively, whereas the non-encapsulated CEO released 100% within 6 hours (Ibrahim *et al.* 2022). Additionally, diluted geraniol-in-water microemulsions were effective against Asian tiger mosquitoes, maintaining activity for up to 3 hours at a dose of 190 µg/cm^2 of active ingredient. These low-viscosity formulations with monodispersed oil droplets of small hydrodynamic diameter proved suitable for essential oil delivery. Spectroscopic analysis revealed a compact outer configuration and a flexible inner structure, enhancing the encapsulation system's performance (Chatzidaki *et al.* 2022).

Encapsulation using chitosan serves to shield active compounds from environmental degradation over a defined period. Over time, numerous encapsulation

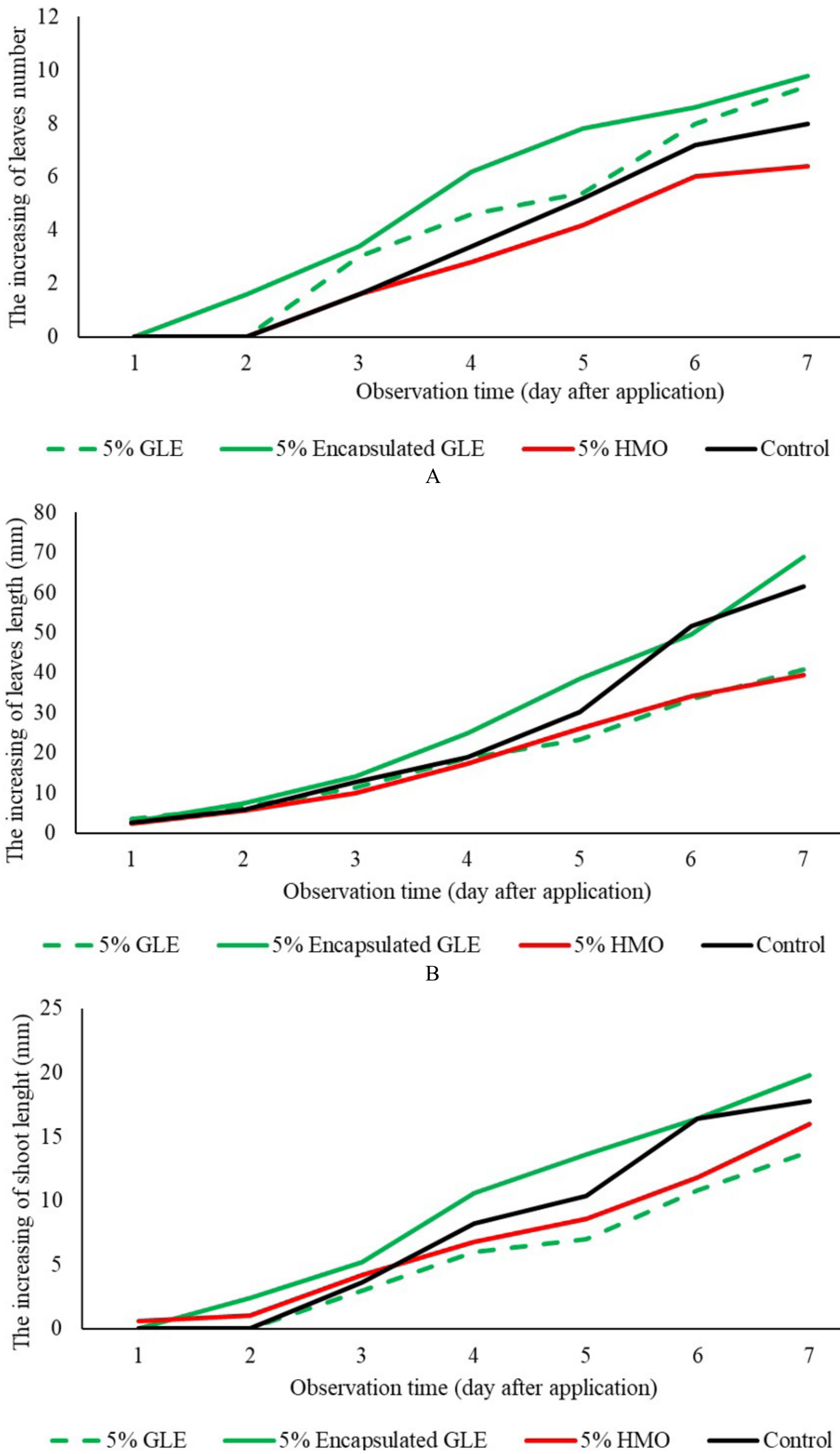

Figure 2. The increase of citrus leaves number (A), leaves length (B), shoot length (C) after application by 5% GLE, 5% encapsulated GLE, 5% HMO, and control (%) during a week of observation

strategies have been designed to entrap various bioactive substances—such as therapeutic drugs, essential oils, hemoglobin, and vaccines—by utilizing chitosan as a protective shell. Techniques such as emulsification, spray drying, and coacervation are commonly employed to achieve this encapsulation. The release of the core materials from the chitosan matrix may occur through several pathways, including diffusion, dissolution, melting, or structural rupture (Raza *et al.* 2020). Owing to its biodegradable, biocompatible, and versatile nature, chitosan has consistently demonstrated its efficacy as an ideal shell-forming biopolymer for delivering a wide array of active ingredients.

Table 2. Recapitulation of analysis of variance for 5% GLE, 5% encapsulated GLE, 5% HMO treatments, and control

Characters	Mean square	CV (%)
Citrus leaves number	11.87^{ns}	50.96
Citrus leaves length	1106^{ns}	60.59
Citrus buds length	32.72^{ns}	53.62

ns: not significant (P<0.05)

Microencapsulation is a technique designed to encase bioactive compounds within microscopic capsules, enabling their stabilization and controlled release under favorable environmental conditions. This method not only facilitates the conversion of liquid formulations into dry, free-flowing powders, but also minimizes particle agglomeration, thereby enhancing storage stability. Encapsulated compounds are effectively shielded from degradation factors such as oxidation, thermal stress, pH fluctuations (acidic or alkaline), humidity, and volatilization. Moreover, encapsulation reduces the likelihood of undesired chemical reactions, such as those that may lead to decomposition or polymerization of sensitive

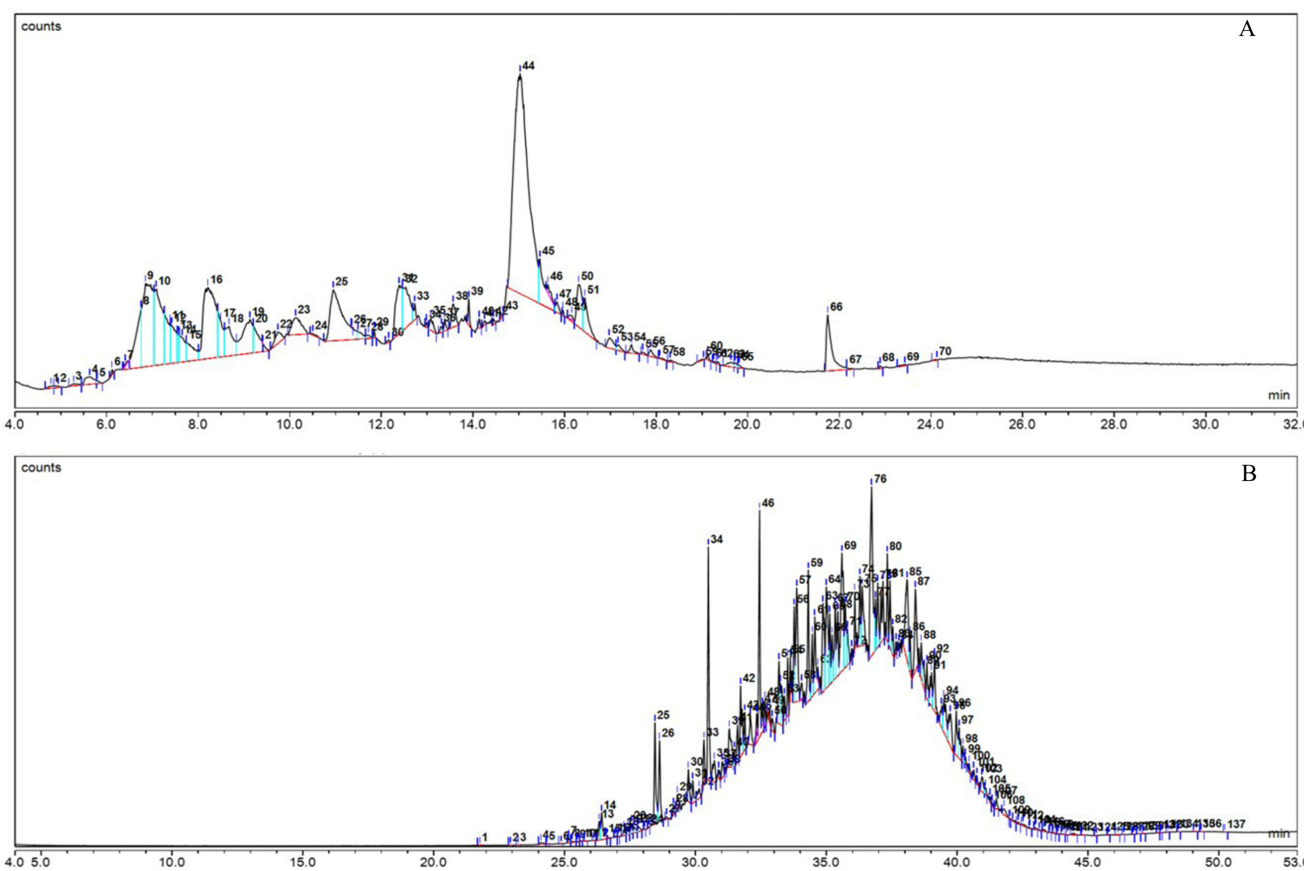

Figure 3. (A) GC-MS chromatogram of guava leaf extract (*Psidium guava* L.) and (B) horticultural mineral oil

Table 3. The dominant compounds found in HMO and GLE probing by GC-MS

Source	Peak	Real time	Hit# 1	Hit# 2	Hit# 3	Rel.Area (%)
HMO	76	36.73	17-Pentatriacontene	Tetrapentacontane, 1,54-dibromo-	tert-Hexadecanethiol	6.44
GLE	44	15.03	Quinic acid	Lactose	d-Glycero-l-gluco-heptose	29.72

components. Each microcapsule generally consists of a central core—containing the active agent—encased within a surrounding protective wall or shell (Timilsena *et al.* 2019). The efficiency and structural integrity of chitosan-based microcapsules are strongly influenced by several physicochemical parameters of chitosan itself, including its surface charge, particle size, molecular weight, and degree of deacetylation. Due to its remarkable characteristics—such as being biodegradable, non-toxic, and biocompatible—chitosan is widely regarded as a promising encapsulation material. These properties have supported its broad utilization, especially in advanced biomedical applications like targeted drug delivery systems and regenerative tissue engineering (Raza *et al.* 2020).

The present findings reveal that volatile organic compounds (VOCs) emitted by guava can prime and activate defense responses in nearby citrus plants. This includes early defense signaling and the synthesis of defense-related proteins and metabolites, leading to enhanced resistance against herbivores. Citrus plants exposed to guava VOCs showed elevated expression of genes involved in jasmonate (JA) biosynthesis and signaling, along with increased levels of JA and jasmonic acid isoleucine. Key guava VOC components, such as (E)-β-caryophyllene and DMNT, were found to trigger the expression of Lipoxygenase 2 and/or PI-like genes, as well as the accumulation of JA in citrus foliage. Additionally, (E)-β-caryophyllene binds to TOPLESS-like (TPL-like) proteins, influencing TPL-mediated signaling. These findings suggest that guava VOCs act synergistically in pest management—not only by deterring herbivorous insects and attracting their natural enemies but also by enhancing JA-mediated anti-herbivore responses in citrus. Overall, the study contributes in advancing knowledge of plant interactions influenced by volatile compounds and highlights the potential of guava VOCs in ecological pest control within intercropping systems (Ling *et al.* 2022). This priming effect suggests an ecological benefit in intercropping guava with citrus, reinforcing the dual-action mechanism-repellency and induced resistance.

Volatile constituents present in essential oils extracted from several non-host plants can interfere with the host recognition behavior of *D. citri*. The repellent efficacy of these essential oils varies based on the distinct nature and level of their volatile constituents. Several oils, including those from eucalyptus, lemongrass, lavender, and commercial blends containing rosemary, orange, clove, cinnamon, and eucalyptus have been reported to reduce *D. citri* infestations by 58–88% (Hall *et al.* 2018). Essential oil extracts obtained via hydrodistillation from both young and mature leaves of five Brazilian guava cultivars also demonstrated repellent properties, with mature leaves exhibiting stronger repellency (Silva *et al.* 2023). Furthermore, oils derived from Tagetes species and *Foeniculum vulgare* Mill. showed both repellent and toxic effects against *D. citri* nymphs and adults, with toxicity levels demonstrated a positive association with concentration, followed by a time-dependent decline (Mendoza-García *et al.* 2019).

The 5% encapsulated HMO process allows degradation of the paraffin compounds contained in the HMO. In the encapsulation process, it is possible for the volatile compounds in HMO to evaporate and leave low amounts of the compounds. The process of microencapsulation contributes to managing the volatility and release characteristics of essential oils (Singh *et al.* 2022). But in reality the freeze dry process in HMO causes a decrease in the quality of the results. The retention of properties in nanoencapsulated products for a long period can be influenced by several factors such as a) materials used; this should be compatible with the core and protect it from surrounding environment (Cano and Maspoch 2012), b) encapsulation methods; the different encapsulation methods can affect the particle size, morphology and stability of nanoencapsulated product (Pateiro *et al.* 2021), c) the stability of the active compounds can be influenced by factors like temperature, pH and exposure to air, d) release triggers; incorrect choice of release triggers can lead to premature release of the active compounds, reducing their effectiveness and stability (Phanse *et al.* 2022), and e) application methods (Taouzinet *et al.* 2023).

Effect and sign of phytotoxicity on treated citrus leaves was not found. It was indicated that applied treatment to citrus leaves had no effect on the phytotoxicity. Benzoic acid, 3,4,5-trimethoxy-2-nitro- compound was found on GLE using GC-MS. This compound was grouped as salicylic acid (NCBI 2023). Tetrapentacontane, 1,54-dibromo-, octadecanal, 2-bromo-, cholestan-3-ol, 2-methylene-, (3ß,5a)-, 7,8-Epoxylanostan-11-ol, 3-acetoxy-, methyl glycocholate, 3TMS derivative were compound that found on HMO using GC-MS. They were also grouped as salicylic acid (NCBI 2023). Li *et al.* (2022) reported that the phytohormone salicylic acid (SA) not only is a well-known signal molecule mediating plant immunity, but also is involved in plant growth regulation. SA mediates growth regulation by affecting cell division and expansion. The treatment of 5% encapsulated GLE assumed that it could induce the citrus plants growth especially on leaves number, leaves length and shoot length. Among the treatments was not shown significantly different on Table 2. So, the treatment using 5% GLE, 5% HMO and also 5% encapsulated GLE did not affect citrus growth and development.

GC-MS method was conducted to identify various secondary metabolites or compounds present in the plant extract (Figure 3). Three dominant compounds on GLE were quinic acid, lactose and d-Glycero-l-glucose-heptose. Quinic acid is handled as a phenolic in the current topic due to metabolic factors despite lacking an aromatic ring or phenolic group. It was characterised as "polyphenols" an unfortunate term since not all of its derivatives are polyhydroxy (Robards *et al.* 1999). Sing *et al.* (2021) was reported that polyphenols typically exhibit anti-feedent and anti-deterrent effects on most of the insects. Lactose was carbohydrates or phenols compounds on the nutritional profile of GLE. L-Glycero-D-gluco-heptose is a hexadecanoic acid that is used as a synthetic intermediate. It was included in carbohydrate group compounds.

There were desulphosinigrin compounds found on GLE that have potential as repellent. Maliza *et al.* (2023) reported that desulphosinigrin creates hydrophobicity and hydrogen interactions with the OBP1 target protein that are utilised in in-silico research showing this compound potential as repellent for insects. Some volatiles released by the GLE had a protectant and repellent effect against the Asian citrus psyllid (*Diaphorina citri*) such as desulphosinigrin and quinic acid. Dimethyl disulfide (DMDS) is also believed to exhibit repellent properties against *Diaphorina citri*. This compound acts as an insecticidal and defensive volatile, produced specifically by guava plants in response to physical damage. DMDS is rapidly generated upon tissue disruption (Rouseff *et al.* 2008). 17-Pentatriacontene, Tetrapentacontane 1,54-dibromo-, tert-Hexadecanethiol were dominant compounds found on HMO. They had pharmaupetical activities as antimicrobial bioactive compounds (Sivakumar 2014; Oviya *et al.* 2022; Jahajeeah *et al.* 2023). Another volatil compounds on HMO were ethyl iso-allocholate, cholestan-3-ol, 2-methylene-, (3ß,5a)-, cholestan-3-ol, 2-methylene-, (3ß,5a)-, 7,8-Epoxylanostan-11-ol, 3-acetoxy-, spirost-8-en-11-one, 3-hydroxy-, (3ß,5a,14ß,20ß,22ß,25R)-, and 25-Norisopropyl-9,19-cyclolanostan-22-en-24-one, 3-acetoxy-24-phenyl-4,4,14-trimethyl- included to steroid groups. The 7,8-Epoxylanostan-11-ol, 3-acetoxy- was most dominant found and its compounds showed a property as insect repellent (Musman *et al.* 2020).

In conclusion, encapsulation significantly enhanced the repellent effect and persistence of 5% Guava Leaf Extract (GLE), effectively reducing the presence of *Diaphorina citri* on citrus leaves. This method improved the duration of GLE's repellent action. However, encapsulation did not have a similar effect on Horticultural Mineral Oil (HMO); instead, it reduced HMO's ability to repel *D. citri* from feeding. Additionally, no phytotoxicity was observed on citrus leaves.

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