

Incorporation of essential oil from *Vitex gardneriana* (Lamiaceae) in microemulsions systems based on mineral and cottonseed oils increased its bioactivity against a coconut pest mite

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ABSTRACT

Coconut (*Cocos nucifera* L.) is an important cash crop for Asia, Africa and tropical America. The coconut mite, *Aceria guerreronis* Keifer (Acari: Eriophyidae) is a major coconut pest, inflicting heavy damage to fruits, thereby reducing yields and farmers profitability. Previous research has demonstrated that high contents of sesquiterpenes compounds present in the essential oil of *Vitex gardneriana* Schauer (Lamiaceae) (EOVG) showed high potential to control *A. guerreronis*. Systems stabilized by surfactants, such as microemulsions (MEs), are promising to carry essential oils due to their solubilization and protection properties, generally allow for greater spreadability, wettability and have thermodynamic stability and lower viscosity. Here, we compared the acaricidal activity of MEs containing EOVG a based cottonseed oil (CO) and mineral oil (MO) as different oily phases. MEs formulations were obtained through the pseudoternary phase diagram using a 1:1 mixture of polysorbate 80: propylene glycol as surfactant and co-surfactant, respectively, and CO and MO as oily phases and water as aqueous phase. The MEs were characterized by polarized light microscopy, dynamic light scattering as well as by rheological behavior. Results based on concentration-mortality bioassays, showed that the toxicity of ME-CO containing EOVG was higher (LC₅₀ = 195 ppm) in comparison with ME-CO without EOVG (LC₅₀ = 669 ppm). Similarly, adding EOVG to ME-MO increased the toxicity further (LC₅₀ = 120 ppm). Moreover, the LC₅₀ of ME with and without EOVG for *A. guerreronis* was higher than the LC₅₀ determined for the EOVG alone (LC₅₀ = 888 ppm). The MEs containing EOVG in their LC₈₀ and mainly LC₅₀, repelled the mite. Overall, our results indicate that the MEs hold potential for controlling *A. guerreronis*, especially when EOVG was added with both oil phases.

1. Introduction

Coconut (*Cocos nucifera* L.) is a high-value perennial tropical crop for Asia, Africa and tropical America countries. Coconut cultivation worldwide covers 12.3 million hectares, which annually produce 61.4 million metric tons of coconut fruit (Ignacio and Miguel, 2021). Apart

from the most valuable fruit, the coconut palm is a source of edible products such as coconut water, virgin oil, copra and coconut milk, as well as natural fiber (shell) and activated carbon (walnut shell) (Renoret et al., 2013; Prades et al., 2016; Hidalgo, 2017; Patil et al., 2017).

The coconut mite, *Aceria guerreronis* Keifer (Acari: Eriophyidae), is a serious coconut pest that inflicts heavy damage to fruits. Colonies of this

Abbreviations: EOVG, Essential oil from *Vitex gardneriana*; MEs, Microemulsions; CO, Cottonseed oil; MO, Mineral oil.

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mite develop underneath the bracts of young fruit feeding on tissue cells and leading to yellowish-white triangular patches that become necrotic (Haq, 2011; Monteiro et al., 2012; Navia et al., 2013; Teodoro et al., 2014). The attack of this mite leads to reduction in fruit size, premature fall, weight loss and value depreciation for fruit intended for water market (Wickramananda et al., 2007; Navia et al., 2013; Teodoro et al., 2014). This pest is usually managed in Brazil through preventive spraying of pesticides (Oliveira et al., 2017), which may lead to problems like pest resistance, environmental contamination, outbreaks of secondary pests, mortality of natural enemies and risks to human health (Guedes et al., 2016; Lima et al., 2016; Oliveira et al., 2017; Monteiro et al., 2018).

Essential oils derived from plants are promising for containing secondary metabolites with proven toxic and repellent activities against arthropods (Isman, 2000, 2016; Pavela and Govindarajan, 2017) in addition to being generally environmentally friendly (Akhtar and Isman, 2012; Miresmailli and Isman, 2014). The *Vitex* genus (Lamiaceae) produces a variety of secondary metabolites that include ecdysteroids and terpenoids, some of which have demonstrated insecticidal and repellent properties (Klein, 2004; De Sena Filho et al., 2008, 2017). In addition, a diversity of sesquiterpene backbones is reported in *Vitex* genus (Barreto et al., 2021). Indeed, *Vitex gardneriana* Schauer (Lamiaceae) is an endemic species of the caatinga biome and produces essential oils rich in sesquiterpenes (yield of 0.33%), mainly, 6,9-guaiadiene (19.3%), carophyllene oxide (18.6%), *L*-calamenene (13.9%), with proven toxicity against *A. guerreronis* (De Sena Filho et al., 2017). However, as observed for other essential oils, their volatility and chemical instability in the presence of air, light, humidity, and high temperatures compromise its persistence in the environment, requiring a greater number of applications. In addition, their immiscibility in water prevents its direct use (Ferreira et al., 2015; Souza et al., 2016; Shao et al., 2018). Therefore, systems stabilized by surfactants, such as microemulsions (MEs), can be used to overcome these limiting properties of essential oils (Ferreira et al., 2015). In fact, MEs containing essential oils have superior control efficiency when compared to encapsulation-free essential oils (Xu et al., 2010; He et al., 2016; Navayan et al., 2017; Wang et al., 2017).

MEs have several advantages such as thermodynamic stability, dispersion of droplets on a nanometer scale, solubilizing of active hydrophobic compounds in water, such as essential oils with the aid of surfactants and/or co-surfactants, which enables the reduce surface and interfacial tension, maintaining the dispersed system. In addition, the incorporation of essential oils into the internal phase of these systems preventing flocculation of the active ingredient, chemical degradation, volatility and improving the efficiency of essential oils (Fanun, 2012; Ferreira et al., 2015; Kale and Deore, 2017; Shao et al., 2018).

Vegetable oils and mineral oil (MO) have high content of lipophilic compounds and thereby can be used to form MEs aiming at agricultural pest control (Buteler and Stadler, 2011; Scamorosenco et al., 2020; Anicescu et al., 2021). Previous studies of our research group comparing toxic effects of vegetal oil shows the use of CO was lethal and repellent against *A. guerreronis* (Teodoro et al., 2017). CO is the main byproduct of cotton (*Gossypium* spp.) processing, representing approximately 17–27% of the seed weight. Cotton is one of the most important oilseed crops in the world (Suo et al., 2021). MO, in turn, is largely used for controlling an array of agricultural pests (Buteler and Stadler, 2011).

In this context, we aimed at developing MEs containing EOVG a based CO and MO as oily phases and evaluate their toxic and repellent activities against *A. guerreronis*. Our approach explored the formulation of water-based MEs containing EOVG and synergistic oil phases as an ecological alternative to pesticides.

2. Materials and methods

2.1. Materials

The surfactant and co-surfactant used were respectively polysorbate

80 (NEON) and propylene glycol (Êxodo Científica). The CO was purchased from HG Comercial LTDA (Aracaju, Sergipe, Brazil) and the MO was acquired in the local commerce. The high-purity water was prepared with a Millipore Milli-Q Plus purification system. EOVG was extracted by hydrodistillation following procedures previously described (De Sena Filho et al., 2017). Adults of *A. guerreronis* were obtained from fruit of a green dwarf coconut plantation located in the city of Aracaju (10° 57'S; 37° 03'W), state of Sergipe, Brazil. Although we did not determine the ages of the mites, all individuals used were collected from colonies in the initial stage of oviposition as an age-standardization effort (Oliveira et al., 2017).

2.2. Preparation of microemulsions and construction of pseudoternary phase diagrams

Two pseudoternary phase diagrams using either CO or MO as oily phases and the (1:1) polysorbate 80 and propylene glycol were obtained using the water titration method as previously described (De Sá et al., 2020). Initially, a blend of surfactant and cosurfactant (S/CoS) were mixed in a 1:1 (v/v) ratio for 12 h. Afterwards, the oil phase and the S/CoS dispersion were homogenized in the following proportions: 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8 and 1:9 with a magnetic stirrer (Martemag15) for 30 min. Each specific mixture was further titrated with distilled water (dropwise) under magnetic stirring for another 30 min. All formulations were held for 5 days at room temperature, and samples were observed for visual appearance and microscopic appearance using polarized light microscopy to obtain a pseudoternary phase diagrams. Transparent systems were classified as MEs. The ME region was highlighted in pseudoternary phase diagrams using Origin 8 software.

Two formulations were selected from the ME region of each diagram. Afterward, a concentration of 2% of the essential oil was added to new MEs, respecting the maximum oil phase limit of the selected MEs. Each formulation remained in equilibrium for five days for further characterization. Selected formulations and those with the addition of EOVG were characterized by polarized light microscopy, dynamic light scattering and rheological measurements.

2.3. Polarized light microscopy (PLM)

The isotropic behavior of the formulations was evaluated at room temperature through PLM. Briefly, a drop of each ME sample was placed between a coverslip and a glass slide and then examined under polarized light using an Optical Olympus BX-51 Microscope equipped with a digital camera LC Color Evolution (PL-A662). Photomicrographs were taken at $\times 20$ magnification.

2.4. Dynamic light scattering (DLS)

Droplet size and polydispersion index (PDI) of MEs was measured after dilution of the sample with high-purity water in rates of 1:150 (v:v) using a dynamic light scattering method by Zetasizer Nano-ZS instrument (Malvern Instruments, Worcestershire, United Kingdom) (Souza de Araujo et al., 2020).

2.5. Rheological measurements

The formulations rheological analysis was performed using a Discovery Hybrid Rheometer (DHR-1, TA instruments) as described by (Souza de Araujo et al., 2020). A cone and plate device (50 mm diameter, 1° cone angle and 200 μm gap) was used for rheological characterization of the MEs. Samples were carefully applied to the lower plate, ensuring that formulation shearing was minimized, and allowed to equilibrate for at least 3 min prior to analysis. All rheological determinations were carried out on all samples at $25 \text{ }^\circ\text{C} \pm 0.2 \text{ }^\circ\text{C}$. Steady shear rate sweep experiments (flow tests) were performed with shear rates ($\dot{\gamma}$) in the range of $0\text{--}200 \text{ s}^{-1}$. The shear rate region used was

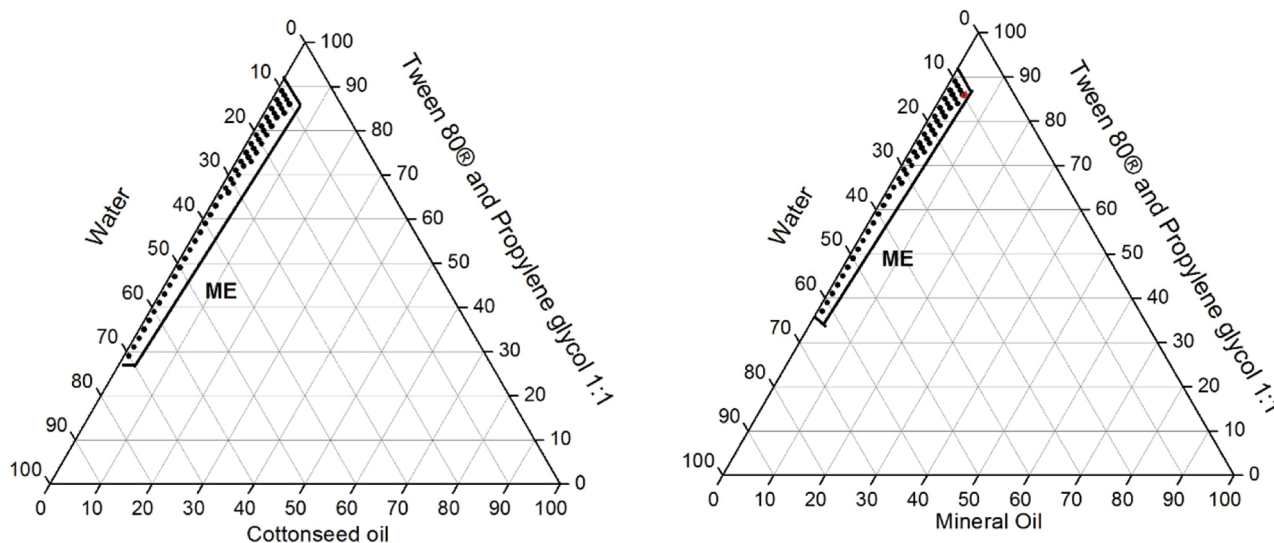


Fig. 1. Phase behaviors of Tween 80/ propylene glycol/CO or MO/water.

selected on the basis of the strength of resistance to the applied stresses. Shear viscosities (η) were determined by Newtonian model ($\sigma = \eta \cdot \dot{\gamma}$), using TA Instruments TRIOS software.

2.6. Toxicity

MEs were sprayed through a Potter tower (Burkard, United Kingdom) at a pressure of 0.34 bar (34kPa) with a 1.7 mL aliquot, which resulted in a residue of 1.8 ± 0.1 mg/cm² in accordance with standard guidelines (Hassan et al., 1994). The arenas (experimental units) consisted of 1 cm diameter perianth discs of young coconut fruits sitting in Petri dishes (100 × 15 mm) and immersed in a mixture of 5% agar, 0.3% methylparaben (Nipagim®) as a fungicide and distilled water. The arenas were opened with the help of a mold to expose the plant tissue. The concentrations of MEs with and without essential oil were selected after preliminary bioassays and conducted over a wide range, allowing the selection of the highest concentration that did not kill and the lowest concentration that killed all mites. The selected MEs were diluted in distilled water to obtain five increasing concentrations of ME containing MO without EOVG (A1) (120, 200, 300, 600, 1000, 1200) and five increased concentrations of ME containing MO with EOVG (A3) (60, 90, 200, 600, 800 ppm). On the other hand, five increasing concentrations of ME containing CO without EOVG (A2) (400, 700, 900, 1300, 1500 ppm) and five concentrations of ME containing CO with the presence of EOVG (A4) (70, 200, 400, 800, 1000 ppm) were assayed.

The sprayed discs were dried in the open air for 30 min before 20 adults of *A. guerreronis* were placed in each. Ten repetitions, totaling 200 mites per concentration, were conducted. The arenas were covered with a black fabric to simulate darkness conditions underneath fruit bracts. Petri dishes were kept in a controlled chamber at 27 ± 3 °C, with a relative humidity of $70 \pm 10\%$ and a 12 h photoperiod. The numbers of living and dead mites were recorded 24 h after sprayings and mites were considered dead when they did not move after prodding with a fine paintbrush (Stark et al., 1997).

2.7. Repellency

The procedures used in this bioassay were as described by (Dos Santos et al., 2019). Briefly, two layers of waterproof tape were used to cover half of the coconut perianth discs (1 cm in diameter) before spraying with the respective LC₅₀ or LC₈₀ of the MEs (A1, A2, A3 and A4), as estimated for *A. guerreronis* in the previous bioassay. The tape was removed and the discs were air-dried for 30 min before a single

adult mite was gently transferred to a tiny drop of dry glue placed in the center of disc. The experimental units were maintained under controlled conditions (28 ± 2 °C, $80 \pm 10\%$ RH and 12-hour photoperiod). The position of the mites in the treated or untreated half of the discs was recorded 1, 24 and 48 h after spraying. Each treatment was repeated five times with 20 mites per replication.

2.8. Statistical analyses

A. guerreronis mortality data were corrected in relation to control using the Abbott formula (Abbott, 1925). Concentration-mortality curves were calculated using Probit analyzes to determine the lethal concentrations (LC) of MEs with and without EOVG towards *A. guerreronis* using the SAS PRO PROBIT procedure (SAS Institute, 2008). The toxicity of the EOVG and the MEs were compared based on LC₅₀ confidence intervals (CI) overlapping. The repellency of MEs to *A. guerreronis* were determined by frequency analysis according to the chi-square test using Procfreq from the SAS software (SAS Institute, 2008).

3. Results and discussion

3.1. Phase behavior of the microemulsion

The phase diagrams constructed using CO and MO as the oil phase, polysorbate 80 and propylene glycol as S/CoS and water is shown in Fig. 1. The ME regions were marked and present a narrowing at the interface with a higher amount of surfactant and less water. Polysorbate 80 is one of the most used non-ionic surfactants for the formulation of EO-based colloidal systems (Ghosh et al., 2013; Bhargava et al., 2015; Balasubramani et al., 2017; Pavoni et al., 2020). This molecule has a non-toxic nature and has influenced droplet size, size distribution and PDI value (Dai et al., 1997; Ghosh et al., 2014; Pavoni et al., 2020). The mixture of polysorbate 80 and propylene glycol were previously used as surfactants to obtain ME (De Sá et al., 2020; Pumival et al., 2020; Souza de Araujo et al., 2020), mainly in regions with a higher proportion of surfactants. This is interesting as the final product will have a high concentration of surfactant, which will be diluted for application. They seem to be more suitable compared to those with very low or very high HLB because they are more likely to move around in the interface (Chang and McClements, 2014). Short chain glycols such as propylene glycol was used for their ability to increase the fluidity of the interface due to the presence of fluidizing groups such as unsaturated bonds, then

Table 1

Droplet size, polydispersity index (PDI), viscosity (η) and coefficient of determination (R^2) of formulations with and without the essential oil of *V. Gardneriana*. Values correspond to the average of 3 replicates (mean \pm standard deviation).

Samples	Size (nm)	PDI	η (Pa.s)	R^2
ME-MO (A1)	11.85 \pm 0.08	0.200 \pm 0.005	0.3928	0.999
ME-CO (A2)	32.00 \pm 0.36	0.180 \pm 0.012	0.4366	0.998
ME-MO + EOVG (A3)	12.52 \pm 0.15	0.153 \pm 0.55	0.3387	0.997
ME-CO+EOVG (A4)	34.00 \pm 0.45	0.192 \pm 0.025	0.4153	0.998

demolish the crystalline or liquid gel structure and alter the HLB value to cause formation spontaneous of ME (Kale and Deore, 2017). In addition, the same co-surfactants, when penetrating the surface-active film at the interface, lead to a decrease in the flexural modulus of this film, favoring the formation of water-dilutable MEs (Pavoni et al., 2020).

The two diagrams (Fig. 1) had the same behavior when compared to mixtures of S/CoS, water and their different oily phases. MEs were found in the region of 9.9: 0.1–9.6, 0.4 varying 1% of oily phase. All formulations obtained from this region were fluid, isotropic, transparent and/or translucent. Other systems obtained from the rest of the diagrams were emulsions and phase separation, which are not presented here.

3.2. Selection of formulations and incorporation of the essential oil

Dots in Fig. 1 indicate the MEs obtained in this study. Two MEs containing 4% oil phase, 10% water and 86% S/CoS were selected from each diagram. These MEs were chosen because they present the highest concentration of the oil phase, considering the incorporation of the essential oil and the dilution of the final product for application, thus causing less structural changes in the formulations. The EOVG was incorporated in the selected MEs in concentration of 2% (v/v), respecting the maximum limit of 4% of the oil phase, giving rise to formulations A3 and A4. The incorporation of the oil apparently did not change the visual aspect of the MEs as the formulations with presence of EOVG were homogeneous, isotropic and optically transparent. Therefore, MEs with and without EOVG were characterized.

3.3. Microemulsions characterization

3.3.1. PLM

The analysis of MEs (A1, A2, A3 e A4) by PLM did not detect birefringence, which indicated that they showed the same optical properties in all directions. In addition, the photomicrographs resulted in dark field formation for all formulations. In contrast to anisotropic liquid crystals, isotropic materials such as MEs will not interfere with polarized light (Friberg, 1990) and the field of view remains dark. That occurs when the structures formed in the system are smaller than the wavelength of the visible light, making impossible the formation of images (Kreilgaard, 2002). One of the main characteristics of the ME is its optical isotropy, i. e., the refractive index is the same in all directions regardless of the incident light (Patel et al., 2013).

3.3.2. DLS

The MEs were translucent, homogeneous with small variations in droplet size ranging from 11.85 to 34 nm (Table 1), which is within the standard ME scale, ranging from 5 to 50 nm (Sharma et al., 2019). Probably, these small variations are caused by the solubilization of the EOVG by the droplets of the internal phase. Under appropriate conditions, aqueous surfactant solutions are capable of dissolving relatively high amounts of oil (in comparison to the solubility of oil in pure water) by means of solubilization, that is, by incorporating oil molecules into aggregates of surfactants (Christian and Scamehorn, 1995). The kinetic description of oil solubilization in these systems involves the dissolution of oil molecules in the aqueous phase and their subsequent capture by

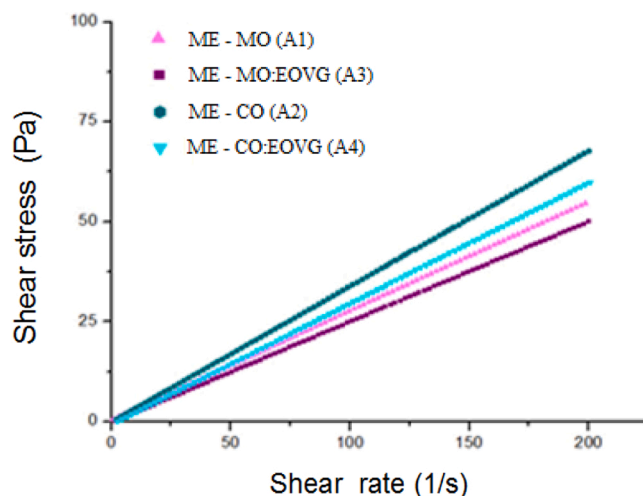


Fig. 2. Rheograms of microemulsions systems (A1, A2, A3 and A4) in the presence and absence of EOVG.

the micelles, which are diffuse in the vicinity of the oil-water interface (Julian McClements and Dungan, 1995; Kabalnov and Weers, 1996).

MEs produced showed PDI varying from 0.153 to 0.2. PDI is the measurement of homogeneity of particles, and it ranges 0.0–1.0 (Pant et al., 2014). Lesser the PDI value has been associated with a mono-dispersed system, with high homogeneity in the droplet population, whereas high PDI values mean a wider size distribution (Gaumet et al., 2008). As a parameter of stability, the particle size and the PDI are relevant for emulsified systems (Li et al., 2010). Small droplet size provides an increased stability against sedimentation and, generally, against flocculation and coalescence (Pons et al., 2003). System stability in nanoscale is related to the how small the essential oil particles are and the presence of substances with low molecular weight, such as aromatic compounds (Lovelyn and Attama, 2011; Duarte et al., 2015). Therefore, the addition of EOVG did not interfere in the shape and size of the droplets, may indicates a good stability for the formulations (Gumiero and da Rocha Filho, 2012).

3.3.3. Rheological measurements

The viscosity and rheological behavior of each system were determined by studies of flow properties, which consisted in determining the flow/deformation properties of the material when subjected to shear stress. The rheological profiles of MEs were defined through the correlation between the rate and shear stress (Fig. 2). The tested MEs showed a linear relationship between shear rate and shear stress represented by an ascending line with a high coefficient of determination ($R^2 > 0.99$)

Table 2

Estimated lethal concentrations (LC_{50} and LC_{80}) of the microemulsions without and with EOVG against *A. guerreronis* after 24 h of exposure.

Samples	LC_{50} (ppm) (95% CI)	LC_{80} (ppm) (95% CI)	χ^2	P
EOVG*	857.73 (670.69–1150.98)	4.806.34 (3.092.20–9.051.48)	3.17	0.20
ME-MO (A1)	365.16 (330.22–402.64)	794.48 (703.51–917.85)	1.62	0.80
ME-CO (A2)	669.18 (623.19–716.76)	1158.00 (1064.00–1281.00)	7.76	0.10
ME-MO+EOVG (A3)	120.49 (100.17–141.78)	393.54 (326.15–496.77)	5.32	0.14
ME-CO+EOVG (A4)	195.27 (162.50–229.14)	617.74 (516.82–768.89)	5.22	0.15

Mean values obtained (n = 8 replicates with 20 adult mites each). CI = confidence intervals, χ^2 = Chi-square, P = Probability (p < 0.05).* De Sena Filho et al. (2017)

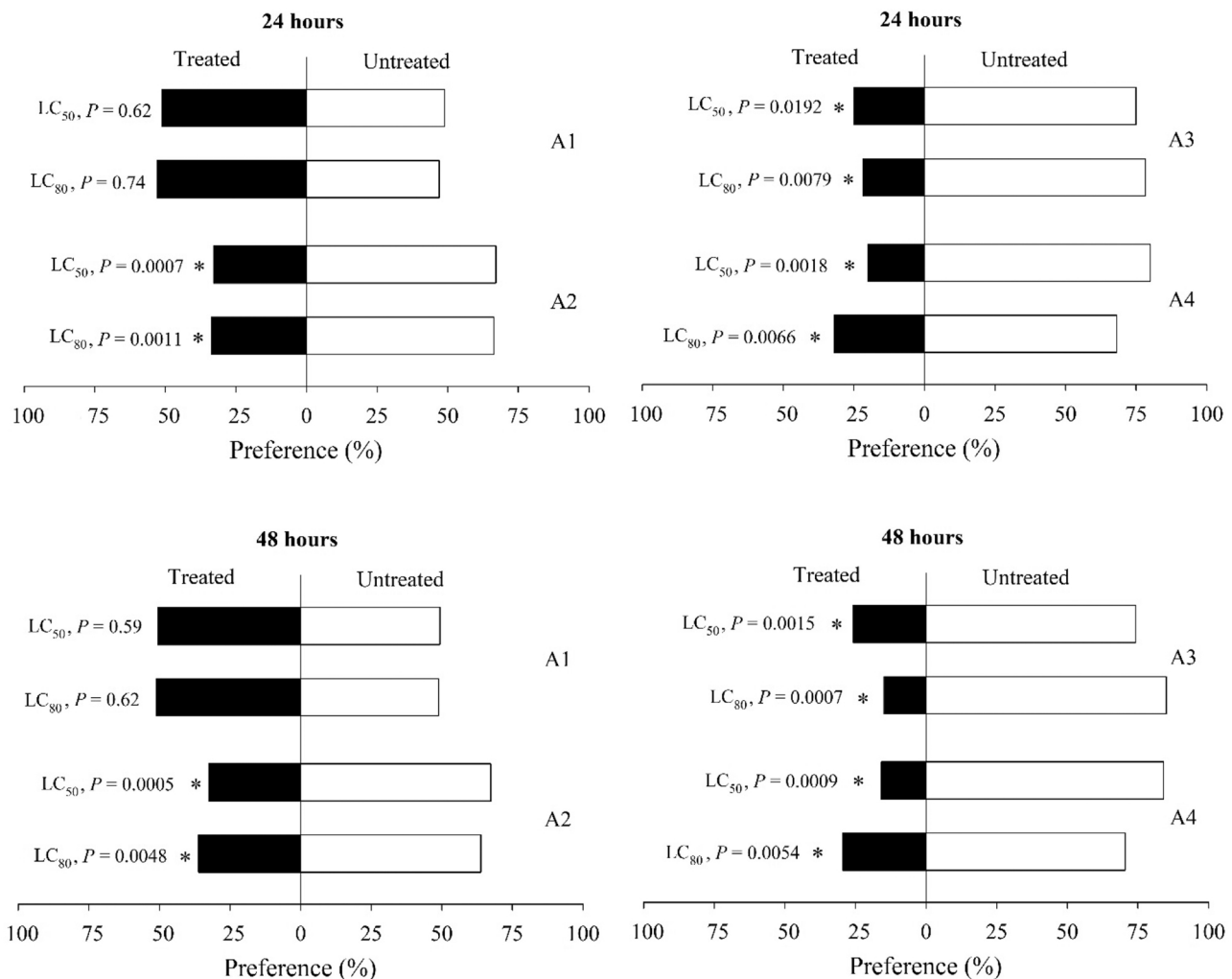


Fig. 3. Preference of coconut mite *A. guerreronis* choosing arena halves sprayed (black bars) or un sprayed (white bars) with LC₅₀ and LC₈₀ of A1, A2, A3 and A4 for the period of exposure of 24 h and 48 h. Each bar corresponds to a mean of five replicates (n = 100 mites). Significance level is given (two-sided binomial test).

indicating the properties of the Newtonian fluid of the MEs (Ge et al., 2014). The Newtonian profile is expected in ME systems due to the small size of the drops and the low interaction between them (Polizelli et al., 2006). Small differences can be noticed between the slopes of the lines, suggesting different values of viscosity (Table 1). The addition of EOVG caused a slight decrease in viscosity. Since the viscosity is the resistance to flow, the addition of essential oil favored the separation between the particles due to its lipophilic nature, further reducing the interaction between them.

3.4. Toxicity

Estimated LC₅₀ and LC₈₀ for the EOVG and all formulations (A1, A2, A3 and A4) are presented in the Table 2. Based on LC₅₀ CI overlapping, all MEs were more toxic to *A. guerreronis* than the EOVG and the MEs containing EOVG (A3 and A4) were significantly more toxic than MEs without this oil (A1 and A2). Moreover, A3 was more toxic to the mite than A4. The LC of MEs incorporated with EOVG (A3 and A4) were about eight fold lower than that reported for free EOVG. The LC of A3 and A4 are significantly lower compared to other oils, botanical extracts and their respective constituents against *A. guerreronis*. For example, LC₅₀₍₉₀₎ for methanolic extracts from leaves of *Genipa americana* L. (Rubiaceae) was estimated as 0.60(16.69)mg.mL⁻¹, equivalent to 600 (16690)ppm (Souza de Jesus et al., 2020). Another study determined the LC₅₀ of the OE of the accession LGRA106 of *Lippia gracilis* Schauer

(Verbenaceae) and its major constituent thymol as 4.28 and 5.34 mg/mL, equivalent to 4280 and 5340 ppm, respectively (Dos Santos et al., 2019). On the other hand, the LC₅₀ of the OE of the accession LGRA109 of *L. gracilis* and its major constituent carvacrol was determined as 28.01 and 6.84 mg/mL, equivalent to 28010 and 6840 ppm, respectively (Teodoro et al., 2021). Also, the LC₅₀₍₈₀₎ of EO of *Citrus sinensis* L. (Osbeck) (Rutaceae) grafted on *Citrus limonia* Osbeck was estimated at 4.28(10.39)mg/mL, equivalent to 4280(10390)ppm, respectively (Brito et al., 2021).

Our results showed that the MEs were able to further enhance the toxicity of the EOVG, which is in line with other studies (He et al., 2016; Navayan et al., 2017; Xu et al., 2010). EOs have low persistence of biological activity, characterized by rapid degradation or evaporation in the environment (Turek and Stintzing, 2013). Pavela and Sedláč (2018) showed the importance of application temperature in the insecticidal effectiveness of EOs, which may vary according to the EOs and their variety in chemical composition, in addition to the mode of application on insects. As observed here, the microemulsion systems were able to encapsulate the chemical constituents of the EOVG, improving its dispersion in water, reducing chemical degradation and volatility. Moreover, MEs are able to reduce the size of oil droplets at the nano-scale, which results in a greater contact surface between the droplets and the target, decreasing their LC₅₀ (Chaisri et al., 2019; Ferreira et al., 2015). Additionally, the superior toxic effect of MEs containing EOVG is possibly related to the ability of the system to reduce contact angles,

allowing the system to be wetted against the hydrophobic surface generating greater spreadability (Giatropoulos et al., 2018; Zhang et al., 2014). Also, there is probably a synergism between the oil phase and the EOVG. Teodoro et al. (2017) reported that CO was toxic to *A. guerreronis*, in addition to being eight times less toxic than pesticides to the predator *Typhlodromus ornatus* Denmark & Muma (Acari: Phytoseiidae), indicating its importance in preserving natural enemies. On the other hand, The mechanism of action of MO is possibly related to cover and suffocation (suffocating effect) of arthropods (Tavemer, 2002).

3.5. Repellence

Except for A1, all formulations repelled *A. guerreronis* including MEs containing essential oil (A3 and A4). The repellency of the LC₅₀ and LC₈₀ of A3 after 24 h are similar, however, after 48 h there is an increase in the LC₈₀ repellency. Probably, with the passage of time, a higher concentration of EOVG retained in the system containing MO will be necessary to continue to repel *A. guerreronis*. The LC₅₀ of A4 formulation repelled *A. guerreronis* up to 48 h. Teodoro et al. (2017) reported that in addition to its toxicity, the CO was able to repel *A. guerreronis* after 1 and 24 h, however, repellent responses varied with time, probably because toxicants suffer temporal degradation. Interestingly, the formulations containing only CO (A2) had prolonged repellency up to 48 h. Apparently, the compounds encapsulated within the MEs are able to retain and avoid the susceptibility of degradation of the compounds to the environment. In addition, when EOVG is added to the system containing CO (A4) there is an increase in repellency in the LC₅₀, probably as a result of synergism between EOVG and CO. The repellent activity of essential oils has been extensively reported in the literature (Benelli and Pavela, 2018; Jokić et al., 2018; Plata-Rueda et al., 2020; Salman et al., 2020) and are related to monoterpenes and sesquiterpenes (Da Camara et al., 2015; Langsi et al., 2020; Ravi Kiran and Sita Devi, 2007). However, the repellence of these compounds are short lived for their volatility (Nerio et al., 2010) (Fig. 3).

4. Conclusions

Sesquiterpenes-rich essential oil from *Vitex gardneriana* microemulsion systems based either on MO or CO increased the acaricidal activity against a key coconut pest mite, *A. guerreronis* reducing eight times the lethal concentration of the free essential oil microemulsified system, in addition to an overall prolonged the repellent effect. This could be related to the protection of sesquiterpenes from volatilization, chemical degradation and synergism with either oily phases. Agricultural industry can benefit from the use of these systems to deliver essential oils due to its efficiency, preservation of volatile compounds in addition to presenting an environmental-friendly formulation to be assayed against a variety of agricultural pests.

Ethical approval

All applicable international, national, and institutional guidelines for the care and use of animals were considered in the present investigation.

CRedit authorship contribution statement

Ighor Costa Barreto: Conceptualization, Methodology, Investigation, Visualization, Formal analysis, Writing – original draft. **Salvana Priscylla:** Methodology, Investigation, Formal analysis. **Adriana de Jesus:** Methodology, Investigation, Formal analysis. **Adriano Pimentel:** Formal analysis. **Victor Hugo:** Methodology, Resources. **Rogéria de Souza:** Conceptualization, Methodology, Resources. **Adenir Vieira Teodoro:** Conceptualization, Methodology, Validation, Resources, Writing – review & editing. **José Guedes de Sena Filho:** Conceptualization, Formal analysis, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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