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Acaricidal Activity of Palm Oil on *Aceria guerreronis* (Acari: Eriophyidae) and a Nontarget Predator[1]

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Abstract The coconut mite, *Aceria guerreronis* Keifer (Acari: Eriophyidae), is a severe and ubiquitous pest of coconut plantations worldwide. Vegetable oils contain fatty acids that are active against a variety of agricultural pests; however, little is known about their efficiency in controlling *A. guerreronis* as well as their adverse effects on its natural enemies. Here, we assessed the chemical profile of palm oil as well as its toxicity and repellence to both *A. guerreronis* and *Typhlodromus ornatus* Denmark and Muma (Acari: Phytoseiidae), a natural enemy of the pest. Oleic, palmitic, and linoleic acids accounted for over 85% of palm oil fatty acid composition. Also, palm oil was approximately 4-fold more toxic to the coconut mite than to its predator. Furthermore, the lethal concentration percentage $(LC)_{50}$ and LC_{99} of palm oil indicated greater activity against the coconut mite than to its predator. Therefore, by exhibiting higher toxicity and repellence to the coconut mite, with substantial selectivity to the predator *T. ornatus*, palm oil is a promising tool to be integrated in the control of *A. guerreronis* in coconut plantations.

Key Words coconut mite, predatory mite, bioactivity, biological control, fatty acids

Plant-derived oils have been shown to exhibit pest control efficacy, to selective favor natural enemies, and to pose little risk to the environment and human health (Isman 2008; Oliveira et al. 2017; Regnault-Roger et al. 2012). These oils contain fatty acids that are bioactive against a variety of pests (Bernklau et al. 2016; Sims et al. 2014) including the coconut mite, *Aceria guerreronis* Keifer (Acari: Eriophyidae) (Oliveira et al. 2017; Teodoro et al. 2017).

Aceria guerreronis is considered a serious pest of coconut, *Cocos nucifera* L., plantations in Africa, Asia, and the Americas (Lawson-Balagbo et al. 2008, Navia et al. 2013). Colonies of this pest develop protected in the fruit perianth and underneath the bracts, and its feeding activity leads to triangular white patches that enlarge and become necrotic, with longitudinal cracks and gum exudation (Navia et

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al. 2013). The necrosis may cover more than half of the surface of the fruit, which may become distorted and stunted, leading to premature fall or a reduction in copra yield and liquid albumen volume (Navia et al. 2013). In the Neotropical region (e.g., Brazil), the management of *A. guerreronis* in coconut commercial plantations relies on preventive and repeated acaricide sprayings, leading to pesticide overuse that potentially causes problems such as human exposure and environmental contamination and outbreaks of secondary pests (Geiger et al. 2011; Guedes et al. 2016). Other potential damage associated with the utilization of synthetic acaricides is their noxious actions on nontarget organisms such as predatory mites (Geiger et al. 2011; Guedes et al. 2016), including members of the family Phytoseiidae, many of which are key natural enemies of the coconut mite (Lawson-Balagbo et al. 2008, Navia et al. 2013). *Typhlodromus ornatus* Denmark and Muma (Acari: Phytoseiidae) has been found foraging on coconut fruits in northeastern Brazil and is possibly associated with natural biological control of the coconut mite (Navia et al. 2005; Reis et al. 2008). This predator is considered a type III generalist which, in addition to small arthropods, feeds on alternative resources such as pollen and nectar (McMurtry and Croft 1997). Because *T. ornatus* is found foraging in coconut fruits, it is important to take into account potential adverse effects to this predator of toxicants used against *A. guerreronis*.

In some Neotropical countries (e.g., Brazil), some vegetable oils, such as the ones extracted from the palm oil, *Elaeis guineensis* Jacquin, could be potentially used against pests as they are cost effective and readily accessible to farmers. However, the potential of palm oil for controlling *A. guerreronis* and its adverse effects on natural enemies has not yet been investigated. Thus, this study was conducted to evaluate the chemical composition, toxicity, and repellence of palm oil to the coconut mite and its predator *T. ornatus*.

Materials and Methods

Oil extraction and trans-methylation. Palm oil (Yao, Bahia, Brazil) was acquired by pressing the mesocarp of mature fruits. Briefly, aliquots (100 mg) of palm oil were weighed into 2-ml amber GC vials to which 1 ml of hexane (Honeywell, Riedel-de Haën, MI) was added before vortexing for 1 min. To form fatty acid methyl esters, 400 μl of 0.5 KOH in a methanol base (Honeywell) was added to the vials and heated to 70°C for 10 min while being vortexed every 2 min. One milliliter of the solution and 1 ml of hexane were added after cooling, and the content was vortexed. After partitioning the organic phase, 1 ml was transferred to another gas chromatography autoinjector vial (Teodoro et al. 2017).

Chromatographic conditions. We used a Thermo Scientific Trace 1310 (GC) fitted with Thermo Scientific ISQ single quadruple (QD) mass spectrometry (MS) and Xcalibur software. Molecules were separated with a Thermo TR-FAME capillary column (70% cyanopropyl polysilphenylene-siloxane, 60 m × 0.25 mm × 0.25 μm film thickness). The column temperature was 45°C for 2 min, increased to 175°C at 10°C/min, held for 20 min, then heated to 215°C at 5°C/min. The MS data were acquired in full scan mode (*m/z* of 50–400) at a scan rate of 0.2 scan/s using electron ionization at 70 eV. The injector temperature was 250°C and the ion source temperature 200°C, and gas chromatographic (GC) analyses were conducted using

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a Perkin Elmer (Shelton, CT) Clarus 500 gas chromatograph fitted with a flame ionization detector (FID) and TC Navigator software. The compounds were separated using a Perkin Elmer Elite Plot 5 capillary column (5% diphenyl–95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 0.25 μm film thickness) and N_2 as the carrier gas (1.2 ml/min flow rate). The column temperature program was 120°C for 1 min, increased to 250°C at 4°C/min, then heated to 280°C at 5°C/min. The injector and detector temperatures were 250°C and 280°C, respectively.

Rearing of *T. ornatus* and source of *A. guerreronis*. Stock colonies of the predatory mite *T. ornatus* were established with individuals collected from unsprayed coconut fruits in São Luís City (02°35′03,46′′S, 44°12′32,14′′W), Maranhão State, Brazil. Species identification was conducted using taxonomic keys, and voucher specimens were deposited in the collection of Maranhão State University, São Luís, Brazil. Colonies of *T. ornatus* were kept under standardized conditions (27 ± 3°C, with 70 ± 10% relative humidity and a 12:12 hr L:D photoperiod). Adults of *T. ornatus* were transferred to PVC discs (5 cm diameter) placed on open Petri dishes (9 cm diameter × 1.5 cm depth) filled with distilled water to prevent mites from escaping. Discs were centrally perforated with a pin and glued to the bottom of the Petri dish. Pollen of the castor bean, *Ricinus communis* L., was provided as a food source every other day. Cotton threads under cover slips (18 × 18 mm) were placed on discs as shelter and oviposition sites. Adult females of *T. ornatus* (8–10 d) from the stock colony were used in all bioassays. Adults of *A. guerreronis* used in all experiments were collected from the same coconut plantation as the predator. Although the precise ages of *A. guerreronis* could not be determined, an age standardization effort was conducted by using mites from colonies in the onset of oviposition. All bioassays were performed under the same conditions of temperature, relative humidity, and photoperiod as previously described for rearing the predator.

Toxicity to *A. guerreronis* and *T. ornatus*. Lethal concentrations (LC) of palm oil were estimated for *A. guerreronis* and *T. ornatus* using concentration–mortality bioassays. Palm oil was sprayed through a Potter tower (Burkard, Rickmansworth, U.K.) onto discs (1.0 cm diameter) prepared with the meristematic tissues of young coconut fruits (approximately 2 mo old) placed in Petri dishes (15 cm diameter, 2 cm deep) containing a mixture of 5% agar, 0.3% methylparaben (Nipagim™) as a fungicide and distilled water for *A. guerreronis* or onto PVC discs (5 cm diameter, as previously described) for *T. ornatus*. Oil was sprayed at a pressure of 0.34 bar (34 kPa) with a 1.7-ml aliquot, which resulted in a residue of 1.8 ± 0.1 mg/cm^2 in accordance with International Organisation for Biological Control (IOBC) guidelines. The concentrations were selected following pilot bioassays and carried out across a broad mortality range, allowing the selection of the highest innocuous concentration (no mortality) and the lowest concentration killing the entire population of *A. guerreronis*.

Five concentrations of palm oil for *A. guerreronis* (1.7, 3.4, 8.5, 13.6, 17 μl/ml) and eight concentrations for *T. ornatus* (13.6, 17, 22.1, 25.5, 28.9, 34, 39.1, 42.5 μl/ml) were used in the bioassays. For each concentration, 10 μl of liquid detergent was added as an adjuvant. Sprayed discs (experimental units) were air-dried for 30 min after which 20 adults of *A. guerreronis* or 7 females of *T. ornatus* were placed on them. Six replicates (discs) for either *A. guerreronis* or *T. ornatus* for each oil concentration were used. Control discs were sprayed with distilled water. Mite

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Table 1. Fatty acid profile of palm oil. Peaks account for 96.54% of the area of all chromatographic peaks of palm oil.

Fatty Acids	Retention Time (min.)	Carbon	Palm Oil
Lauric	16.03	C12:0	0.32
Myristic	18.2	C14:0	0.95
Palmitic	21.05	C16:0	26.85
Stearic	25.43	C18:0	6.68
Oleic	26.76	C18:1	44.94
Linoleic	28.9	C18:2	15.81
Linolenic	32.21	C18:3	0.31
Araquidic	32.92	C20:0	0.49
Behenic	40.93	C22:0	0.08
Lignoceric	45.67	C24:0	0.11

mortality was recorded after 24 h exposure, and the mites were considered dead if unable to move. Data were subjected to probit analyses to estimate LC using the PROC PROBIT procedure (SAS Institute 2008). The LC_{50} value for *T. ornatus* was divided by the LC_{50} value for *A. guerreronis*, and the 95% confidence limits of this toxicity ratio estimate were considered different if they did not include the value 1 (Robertson et al. 2007).

Repellence to *A. guerreronis* and *T. ornatus*. The repellence of palm oil was evaluated on either *A. guerreronis* or *T. ornatus*. The palm oil was applied at its LC_{50} (7.52 μl/ml) and LC_{99} (25.01 μl/ml) rates, which were previously estimated for *A. guerreronis* as described above. Palm oil was sprayed through a Potter tower onto discs that were prepared as outlined above, differing in that only one half of each disc was sprayed. The untreated area was covered with two layers of impermeable tape during spraying; the tape was removed after spraying (Oliveira et al. 2017). The discs were air-dried for 30 min before adult females of either *A. guerreronis* or *T. ornatus* were individually placed on a piece of PVC in their centers. The position of the mites on the sprayed and unsprayed disc halves was recorded after 1 and 24 h. For each mite species and LC level, 60 replicates were performed. Frequency analyses using the chi-square test and Proc Freq SAS software (SAS Institute 2008) were used to compare the percentages of mites choosing the sprayed and unsprayed disc halves. Our previous bioassay in this study revealed that the two disc halves were equally chosen by *T. ornatus* ($P > 0.05$).

Results

Fatty acid composition of palm oil. Saturated and unsaturated compounds are present in the palm oil fatty acid profile (Table 1). Specifically, oleic acid (18:1) was

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the major compound (roughly 45%), followed by high portions of palmitic (C16:0) and linoleic (C18:2) acids, while the acids stearic (C18:0), araquidic (C20:0), lignoceric (C24:0), and behenic (C22:0) were present in minor amounts.

Toxicity to *A. guerreronis* and *T. ornatus*. The median lethal concentration of palm oil (LC_{50}) against of *A. guerreronis* (7.52 μl/ml) was approximately 4-fold lower than the LC_{50} (28.89 μl/ml) estimated for the predator *T. ornatus* (Table 2). The LC_{50} and LC_{99} (25.01 μl/ml) of palm oil, as estimated for *A. guerreronis*, corresponded to the LC_1 and the LC_{30} for *T. ornatus* according to probit analyses.

Repellence to *A. guerreronis* and *T. ornatus*. The LC_{50} of the palm oil, as estimated for *A. guerreronis*, repelled this pest only after 1 h of exposure and did not repel the predatory mite *T. ornatus*, regardless of exposure period. Also, the LC_{99} of this oil was repellent to the coconut mite after 1 and 24 h of exposure and repelled the predator only after 1 h of exposure (Fig. 1).

Discussion

Botanical insecticides have been highlighted for pest control because they pose little threat to the environment and to human health in addition to generally presenting considerable selectivity to beneficials (Isman 2008; Oliveira et al. 2017; Regnault-Roger et al. 2012). Here, we show that palm oil was highly toxic to the coconut mite but significantly less harmful to the predatory mite *T. ornatus*. Furthermore, our results showed that the palm oil repelled significantly more coconut mite than did its natural enemy, *T. ornatus*.

It has been proven that the toxicity of fatty acids and their salts (soaps) against arthropods increase with the chain length, peaking at C10–C12, decreasing at C14–C16, and further increasing in saturated and unsaturated C18 (Bernklau et al. 2016; Sims et al. 2014). Fatty acids exert toxicity by blocking the spiracles of arthropods, causing cuticular ruptures that lead to dehydration (Sims et al. 2014; Szumlas 2002). The fatty acid profile of the palm oil revealed the presence of saturated and unsaturated compounds. The oleic (18:1), palmitic (16:0), and linoleic (18:2) acids were the major compounds of palm oil, accounting for over 85% of its composition. Although oleic and linoleic acids have been shown to be toxic to pests such as the larvae of Mexican corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) (Bernklau et al. 2016), further studies are needed to determine if synergism or antagonism among fatty acids could also play a role in explaining the toxicity of palm oil to the coconut mite.

Based on LC estimates, the coconut mite was roughly 4-fold more susceptible to palm oil than was *T. ornatus*, suggesting compatibility of the oil with this predator. This differential susceptibility could be related to the larger size of the predator relative to the pest mite and the activity of detoxifying enzymes (Kim et al. 2004). The ability to detoxify an acaricide may be related to increasing activities of enzymes such as monooxigenases (Sato et al. 2006), esterases (Anber and Oppenoorth 1989), and glutathione-S-transferases (Fournier et al. 1987).

Arthropods are able to detect toxic substances and move away from the treated area, or the product can cause irritability (evasion after contact) when organisms come into direct contact with the treated area, prompting them to move away (Cordeiro et al. 2010). In this study, palm oil repelled *A. guerreronis* and, to a lesser

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Table 2. Lethal concentrations (LC) in μl/ml and μl/cm^2, with 95% confidence interval in parentheses) of palm oil to the coconut mite *Aceria guerreronis* and to the predatory mite *Typhlodromus ornatus*.

Oils	Slope ± SE	LC	μl/ml (CI)	μl/cm^2 (CI)	*TR*[*]
A. guerreronis ($\chi^2 = 7.73$, df = 3, $n = 600$, $P = 0.052$)	1.044 ± 0.22	LC_{10}	3.88 (1.59–5.54)	0.19 (0.08–0.28)	
		LC_{25}	5.31 (2.85–7.12)	0.27 (0.14–0.36)	
		LC_{50}	7.52 (5.13–9.97)	0.38 (0.26–0.50)	
		LC_{80}	11.62 (8.83–18.42)	0.51 (0.44–0.93)	
		LC_{99}	25.01 (16.47–75.67)	1.27 (0.83–3.85)	
T. ornatus ($\chi^2 = 11.97$, df = 6, $n = 336$, $P = 0.062$)	1.578 ± 0.261	LC_{10}	18.04 (13.83–20.86)	0.91 (0.70–1.06)	
		LC_{25}	22.54 (19.03–25.15)	1.14 (0.96–1.28)	
		LC_{50}	28.89 (25.98–32.33)	1.47 (1.32–1.64)	3.8
		LC_{80}	39.35 (34.73–48.81)	2.00 (1.76–2.48)	
		LC_{99}	67.91 (53.29–109.70)	3.45 (2.71–5.58)	

* TR = Tolerance ratio (LC_{50} to *T. ornatus*/LC_{50} to *A. guerreronis*). TR values are significantly different ($P < 0.05$) if they did not include the value 1.

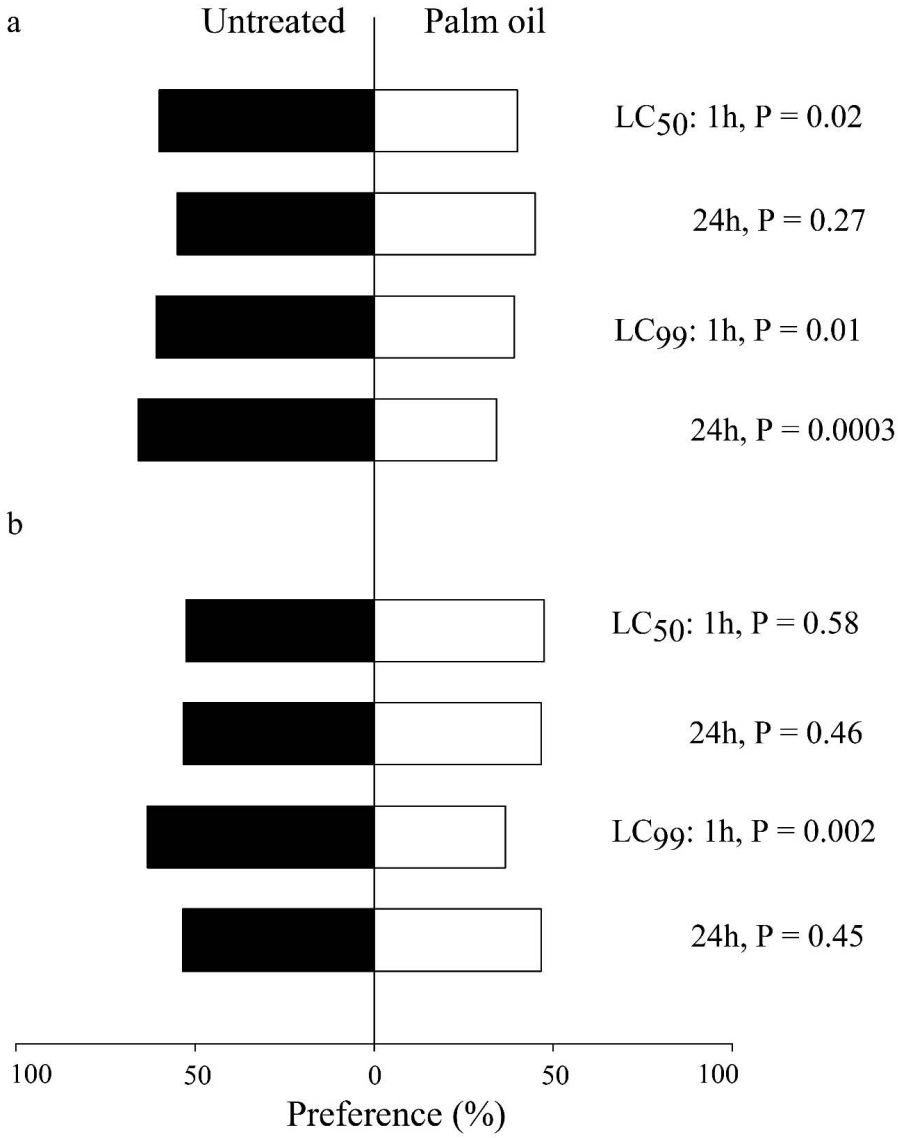

Fig. 1. Percentage of coconut mite *Aceria guerreronis* (a) and its predator *Typhlodromus ornatus* (b) choosing between disc halves that were untreated (black bars) or treated (white bars) with palm oil at its lethal concentration $(LC)_{50}$ and LC_{99}, as estimated for *A. guerreronis*. The period of exposure was 1 and 24 h and each bar corresponds to the mean of 60 mites individually tested. The significance levels are based on frequency analyses.

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extent, its predator *T. ornatus*, indicating the behavioral capabilities of these mites in detecting and avoiding these toxicants. The LC_{50} of palm oil, estimated for *A. guerreronis*, repelled the coconut mite up to 1 h of exposure in contrast with repellence activity at its LC_{99}, regardless of exposure period. This highlights the need for studies assessing the duration of repellence for periods longer than 24 h for LC_{99} of palm oil because toxicants suffer temporal degradation (Fenner et al. 2013). Regarding *T. ornatus*, palm oil repelled this predator only at its LC_{99}, as estimated for *A. guerreronis*, up to 1 h of exposure, indicating selectivity to this natural enemy. Overall, by demonstrating the higher toxicity and repellence to the coconut mite, with substantial selectivity to the predator *T. ornatus*, our findings support palm oil as a promising tool to control *A. guerreronis* in coconut plantations.

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