

Compatibility of degummed soybean and babassu oils with the generalist predatory mite *Typhlodromus (Anthoseius) ornatus* (Acari: Phytoseiidae) preying on *Aceria guerreronis* (Acari: Eriophyidae)

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ABSTRACT

Typhlodromus (Anthoseius) ornatus Denmark & Muma (Acari: Phytoseiidae) is one of the predatory mites associated with the coconut mite *Aceria guerreronis* (Acari: Eriophyidae), considered a severe pest of coconut plantations worldwide. In addition to predatory mites, vegetable oils such as degummed soybean and babassu oils have shown high efficiency in controlling *A. guerreronis*. Here, we evaluated the effects of degummed soybean and babassu oils on the functional response and oviposition of *T. (A.) ornatus* preying on *A. guerreronis*. Exposure to the tested oils did not alter either the functional response or the attack rate of *T. (A.) ornatus*. The variation in prey consumption with the exposure to vegetable oils did not differ from the control, although degummed soybean oil led to higher consumption compared to babassu oil. In addition, prey handling time and peak consumption were negatively affected by the exposure to babassu oil. The number of eggs laid by the female of *T. (A.) ornatus* at intermediate prey density decreased under exposure to degummed soybean oil. Overall, our results indicate that the tested oils are generally compatible with *T. (A.) ornatus* as they do not alter predatory responses but oviposition rate is affected by degummed soybean oil.

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Biological control; coconut mite; fecundity; functional response; vegetable oils

Introduction

The attack of the coconut mite, *Aceria guerreronis* Keifer (Acari: Eriophyidae), is one of the main threats to global coconut production. Colonies of this mite survive in the meristematic region of coconut fruits, under the bracts, where they feed and damage, and in severe stage cause fruit abortion, directly interfering in yield (Negloh et al. 2011; Navia et al. 2013; Oliveira et al. 2017).

To reduce the damage caused by *A. guerreronis*, management strategies with synthetic acaricides have been widely used in coconut plantations. However, pesticides are often inefficiently owed to the hidden habit of this pest (Lima et al. 2015). Moreover, the need for frequent reapplication of these products is generally not economically viable, has the potential to cause negative environmental impacts (Navia et al. 2013) and pressure from developed countries to use new products that combine public health with food security has grown (Isman 2020). Thus, biopesticides have been studied and suggested as an alternative in the management of *A. guerreronis*, especially in organic coconut production systems (Rodrigues et al. 2018; Freitas et al. 2018, 2019; Teodoro et al. 2020).

Recent studies have shown that degummed soybean and babassu oils can be efficient in controlling *A. guerreronis* and have selectivity on the generalist predatory mite *Typhlodromus (Anthoseius) ornatus* (Denmark & Muma) (Acari: Phytoseiidae) (Saraiva et al. 2020), a natural enemy that also inhabits coconut trees (Navia et al. 2013). Although they are considered selective due to the low acute lethality and maintenance of the positive population growth rate of the predator (Saraiva et al. 2020), studies aimed at better understanding the impact of these oils on the rates of

predation and reproduction of *T. (A.) ornatus* are necessary. Haddi et al. (2020) point out that there are few studies on the possible harmful effects of plant-based products on non-target arthropods, which can influence the performance of biological control agents, despite being considered more specific, environmentally safe and more degradable when compared to synthetic acaricides.

The functional response and oviposition of a predator in relation to prey density, characterized by Solomon (1949) and Holling (1959), respectively, are useful in predator-prey interactions and have been used in studies on sublethal effects of acaricides to phytoseiids (Lima et al. 2015; Freitas et al. 2018; Teodoro et al. 2020). Predatory mites can be exposed to pesticides and, even when these organisms survive, physiological and/or behavioural changes may compromise the efficiency of the natural enemy (Desneux et al. 2007; Navia et al. 2013; Lima et al. 2015). This study was aimed at evaluating the effects of degummed soybean and babassu oils on the functional response and oviposition of the predatory mite *T. (A.) ornatus* preying on *A. guerreronis*. *Typhlodromus (Anthoseius) ornatus* is associated with coconut cultivation in northeastern Brazil (Reis et al. 2008), is considered a potential predator of *A. guerreronis*, may consume this pest in a short period of time (Freitas et al. 2018) and is certainly exposed to products used to control *A. guerreronis*.

Material and methods

Mite culture

Stock colonies of *T. (A.) ornatus* were established from specimens collected from coconut fruits of the green dwarf variety at the

Experimental Farm of the Maranhão State University (UEMA), in São Luís city (02°35'03.46"S, 44°12'32.14"W), Maranhão State, Brazil. Approximately 100 females of *T. (A.) ornatus* were transferred to polyvinyl chloride (PVC) (5 cm diameter) discs (arenas) floating on distilled water in uncovered Petri dishes (9 cm diameter by 1.5 cm depth) in order to prevent their escape (Reis et al. 2008). Each disc was centrally perforated with a pin that extended to the bottom of the dish, where it was attached with silicon-based glue, allowing movement of the discs in accordance with the water level. Cotton threads covered with curved transparent PVC (18 x 18 mm) were placed on the arenas to serve as shelter and oviposition sites. The predatory mites were fed *ad libitum* with pollen of castor bean (*Ricinus communis* L.), bee honey diluted to 5% in water, and *A. guerreronis* individuals offered in pieces of coconut perianths. Stock colonies of *T. (A.) ornatus* were maintained in the Laboratory of Acarology (UEMA) under controlled conditions of temperature ($27 \pm 3^\circ\text{C}$), relative humidity ($70 \pm 10\%$), and photoperiod (12:12 L:D).

The adult specimens of *A. guerreronis* used in the experiments were collected in bracts of green dwarf coconut fruits in a plantation located in São Luís (02°38'46"S, 44°13'35"W), Maranhão State, Brazil. Coconut fruits were collected from trees kept free of pesticide application for more than 12 years. Thus, the adult specimens of *A. guerreronis* used in the experiments could not bring biases in the evaluations of the parameters tested in the bioassays described below.

Vegetable oils

Degummed soybean and babassu vegetable oils were selected due to their efficacy against *A. guerreronis* (Oliveira et al. 2017). The methodology for extraction and transmethylation to form acidic methyl esters as well as chromatographic conditions of degummed soybean and babassu oils are described by Oliveira et al. (2017). Moreover, the process to obtain these vegetable oils did not compromise their chemical compositions (Oliveira et al. 2017), and the identifications of their constituents performed through chromatographic analysis are listed below: linoleic and palmitic acids, the main constituents of degummed soybean oil, represent 58.65% and 16.15%, respectively; with the other acids (lignoceric, behenic, arachidic and myristic) participating together in 3.38% of the chemical compounds. Babassu oil has, in greater quantities, lauric, linoleic, myristic, and palmitic acids, which represent 26.16%, 22.64%, 17.91%, and 13.86%, respectively; with the other acids (caprylic, caproic, arachidic, lignoceric, capric and behenic) contributing together with 9.94% of the total characterization of this oil (Oliveira et al. 2017).

Functional response of *T. (A.) ornatus*

The experimental units were composed of PVC discs (7 cm in diameter) placed on Petri dishes and then covered by a mixture of 5% agar, 0.3% methylparaben (Nipagim®) as fungicide and distilled water. An area of 5 cm in diameter was opened with the aid of a hole puncher, exposing the area of the PVC disc over which *T. (A.) ornatus* and *A. guerreronis* were confined. In addition to the agar barrier (1.5 cm high), a hydrophilic cotton barrier moistened in distilled water was placed on the edges of the agar to prevent the mites from escaping (Freitas et al. 2018).

Oil treatments were composed of the lethal concentration 99% (LC₉₉) of babassu oil (29.10 µL/mL; 1.48 µL/cm²) and degummed soybean oil (27.48 µL/mL; 1.39 µL/cm²) estimated for *A. guerreronis* by Oliveira et al. (2017). In the unexposed treatment (control), the experimental units were sprayed with deionized and distilled water. Oils and water were sprayed through a Potter tower (Burkard, Rickmansworth, UK) using a 1.7 mL aliquot at 34 kPa (0.34 bar), rendering a 1.8 ± 0.1 mg/cm² residue, according to the IOBC/WPRS methodology (Hassan et al. 1994). Additionally, 10 µL of neutral liquid detergent (based on sodium lauryl ether sulphate)

was added as an adjuvant in treatments composed of vegetable oils (Teodoro et al. 2017, 2020; Oliveira et al. 2017; Freitas et al. 2018, 2019).

The tests were conducted under the same environmental conditions described above for *T. (A.) ornatus*. For each treatment (babassu oil, degummed soybean oil and control), the following densities of *A. guerreronis* were tested: 10, 80, 110, 200, 300, and 400. Although the exact ages of *A. guerreronis* could not be determined, age standardization was conducted by collecting mites from colonies in the early stage of oviposition, as described in Oliveira et al. (2017). Fragments of coconut perianths (1 cm²) (2 to 3 months of age) containing adult individuals of *A. guerreronis* at the required densities were transferred to the experimental units and their respective treatments. One fertilized female of *T. (A.) ornatus* (6–7 days old) was transferred to each experimental unit, without food deprivation. Each female represented one repetition, in a total of 10 repetitions per prey density. After a period of 24 hours from the transfer of the females of *T. (A.) ornatus*, the number of individuals of *A. guerreronis* remaining in the arena was counted, and the number of prey consumed was obtained by subtraction.

Blank tests were performed for each density of *A. guerreronis* to quantify possible overestimations in prey consumption, mainly because the vegetable oils tested could repel prey, leading to losses by escape. These tests followed the same methodology described above, but without *T. (A.) ornatus*. The amount of prey lost in the blank test was quantified as a correction factor for the consumption of *T. (A.) ornatus* (Lima et al. 2015). As the losses were less than 5%, there was no need for correction in any of the densities.

Oviposition of *T. (A.) ornatus*

The experimental procedures of this bioassay were the same as described in the previous section. The number of eggs laid by *T. (A.) ornatus* females (6–7 days old) that were exposed (Babassu – LC₉₉: 29.10 µL/mL; 1.48 µL/cm²; Degummed soybean – LC₉₉: 27.48 µL/mL; 1.39 µL/cm²) and unexposed (control) to vegetable oils at intermediate density (200 of *A. guerreronis*; n = 10) were counted 24 and 48 h after spraying. At the end of each evaluation, the number of preys consumed was restored to the initial density using pieces of coconut perianths. All evaluations were performed with the aid of a stereoscopic microscope (Stemi 305, Zeiss, Germany). The density of 200 was chosen based on the percentage of consumption (100%) and because it is an intermediate density at which there was the stabilization of consumption for the three treatments.

Statistical analyses

Functional response data were estimated by determining the general shape of the functional response curve based on logistic regression between the mites consumed within each density using the CADMOD procedure in the SAS statistic software (SAS Institute 2008). The cubic model was initially tested due to its capacity to detect the most possible functional response graph variations (Juliano 2001) and the following polynomial function was fitted:

$$\frac{N_e}{N_0} = \frac{\exp(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)}{1 + \exp(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)}$$

where (N_e) is the number of attacked mites, (N_0) is the number of offered density, P_0 , P_1 , P_2 and P_3 are the intercept, linear, quadratic, and cubic coefficients, respectively, related to the slope of the curve. The signs P_1 and P_2 are used to determine the type of functional response. When the linear coefficient is not significant ($P > 0$), it indicates the functional response of type I. When the linear coefficient is significantly negative ($P_1 < 0$), the predator shows a type II functional response. When the linear coefficient is

positive ($P_1 > 0$) and the quadratic coefficient is negative ($P_2 < 0$), the predator shows a type III functional response (Juliano 2001).

As our experiments were conducted with prey depletion (*A. guerreronis*), we used the random predator equation (Rogers 1972; Juliano 2001) as a description of the functional response III:

$$N_e = N_0 \{1 - \exp[(d + bN_0)(T_h N_e - T)/(1 + cN_0)]\}$$

where N_e = number of prey attacked, T = exposure time (24 hours), N_0 = initial prey density, a = attack rate, a constant rate of a successful search, and T_h = handling time. Subsequently, the T_h (handling time) and a (attack rate) parameters of the functional response were estimated with nonlinear least square regression, PROC NLIN procedure, using SAS (SAS Institute 2008), described by Juliano (2001). Attack rate and handling time values were compared using coefficient intervals.

The average variation in prey consumption for each predator at its densities (ΔNa) was calculated according to the equation:

$$\Delta Na = (Na_{N_{max}} - Na_{N_{min}})/(N_{max} - N_{min})$$

where $Na_{N_{min}}$ and $Na_{N_{max}}$ are the minimum and maximum numbers of prey consumed by the predator, respectively, and N_{min} and N_{max} correspond to the minimum and maximum densities (Poletti et al. 2007). Consumption variation data were analysed using ANOVA and were compared using the Tukey HSD test ($P = 0.05$) in SAS software (SAS Institute 2008). The average variation in consumption was plotted as a function of handling time using SigmaPlot 12.5 (Systat Software, Inc., San Jose, USA). Variations close to the control indicated little or no effect on prey consumption by *T. (A.) ornatus*.

The mean oviposition rate of *T. (A.) ornatus* in the periods of 24 and 48 h was analysed by nonparametric Kruskal-Wallis test ($P < 0.05$) using Statistica 12.0 (StatSoft, Inc., Statistica for windows, software-system for data-analyses, version 12.0. Tulsa, USA).

Results

Functional response of *T. (A.) ornatus*

Regardless of the treatment, the females of *T. (A.) ornatus* showed the functional response of type III. This type of response was indicated by the positive and significant linear coefficient ($P_1 < 0.0001$) (Table 1). There was a trend of an increase in the mean number of prey consumed at the lowest prey densities offered (10, 80, and 110), regardless of exposure to the vegetable oils (Figure 1). At densities from 10 to 110, regardless of treatment, approximately 100% of the preys was consumed. Regardless of the treatment, there was a tendency of stabilization in the proportion of prey consumed by *T. (A.) ornatus* at densities from 10 to 200 preys (Figure 2). From the density of 300 prey onwards,

Table 1. Estimated logistic regression parameters of the proportion of adults of the coconut mite *A. guerreronis* consumed by females of the predatory mite *T. (A.) ornatus* unexposed or exposed to degummed soybean or babassu oils. Exposed predators had contact with LC₉₉ of degummed soybean and babassu oils, as estimated for *A. guerreronis*. P₀: intercept; P₁: linear; P₂: quadratic; P₃: cubic; Response: type of functional response.

Exposure	Parameters	Mean ± SE	χ ²	P	Response
Unexposed	P ₀	3.1992 ± 0.6282	25.92	<0.0001	III
	P ₁	0.0462 ± 0.0088	27.35	<0.0001	
	P ₂	-0.00031 ± 0.00003	66.77	<0.0001	
	P ₃	0.00044 ± 0.00004	80.88	<0.0001	
Degummed soybean oil	P ₀	2.9074 ± 0.5669	26.30	<0.0001	III
	P ₁	0.0454 ± 0.0086	27.62	<0.0001	
	P ₂	-0.00027 ± 0.00003	50.31	<0.0001	
	P ₃	0.00003 ± 0.00005	50.45	<0.0001	
Babassu oil	P ₀	1.7938 ± 0.3543	25.63	<0.0001	III
	P ₁	0.0490 ± 0.0054	79.98	<0.0001	
	P ₂	-0.00030 ± 0.00002	144.25	<0.0001	
	P ₃	0.00041 ± 0.00003	156.29	<0.0001	

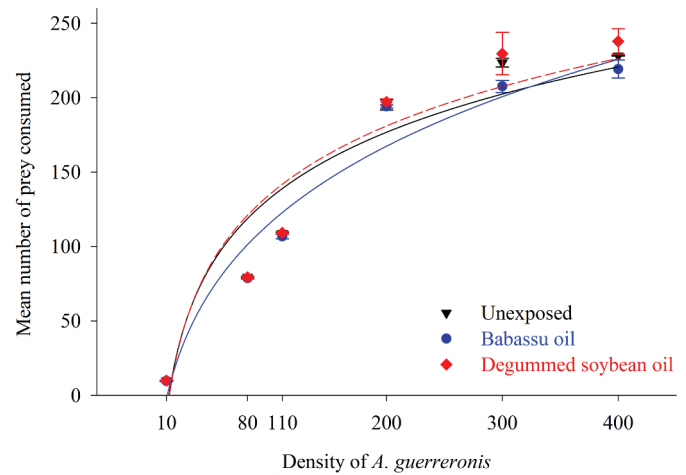


Figure 1. Mean numbers (Mean ± SE) of adults of the coconut mite *A. guerreronis* consumed by adult females of the predatory mite *T. ornatus* unexposed or exposed to degummed soybean or babassu oils as a function of prey density for 24 h. Exposed predators had contact with LC₉₉ of degummed soybean and babassu oils, as estimated for *A. guerreronis*.

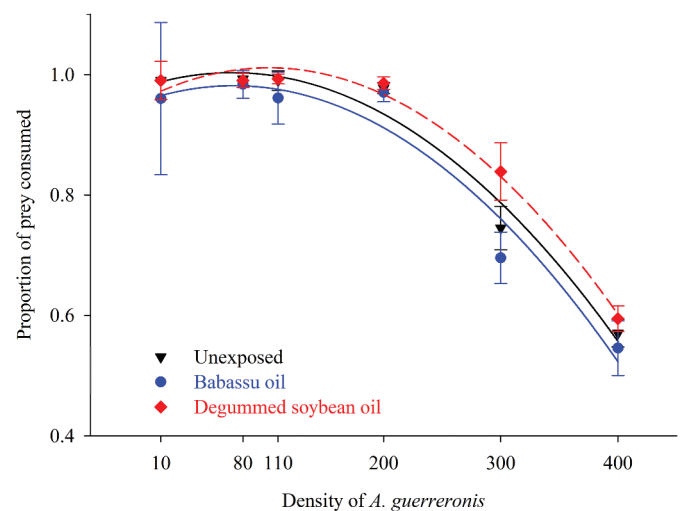


Figure 2. Proportion (Mean ± SE) of adults of the coconut mite *A. guerreronis* consumed by adult females of the predatory mite *T. ornatus* unexposed or exposed to degummed soybean or babassu oils as a function of prey density for 24 h. Exposed predators had contact with LC₉₉ of degummed soybean and babassu oils, as estimated for *A. guerreronis*.

a reduction in the proportion of prey consumed was observed in all treatments (Figure 2).

The attack rate (a) of *T. (A.) ornatus* was not affected by the exposure to the tested oils. In the treatment with exposure to degummed soybean oil, a shorter prey handling time (T_h) was observed, and the opposite occurred with babassu oil, resulting in a longer prey handling time than that observed in the control (Table 2). The peak consumption ($1/T_h$) was significantly higher when there was exposure to degummed soybean oil when compared to babassu oil and control (Table 2). The variation in consumption (ΔNa) was significantly higher for the treatment with degummed soybean oil ($F_{2,27} = 6.12$, $P < 0.0064$) compared to babassu oil, but compared to the control, the exposure to oils caused no differences in the variation of consumption (Figure 3).

Oviposition of *T. (A.) ornatus*

The oviposition of *T. (A.) ornatus* females was lower with the exposure to degummed soybean oil compared to the oviposition of unexposed females ($H = 7.3957$, $df = 2$, $P = 0.0202$, $n = 30$) (Figure 4).

Table 2. Estimates (Means \pm SE and Confidence Intervals) for the parameters attack rate (a), handling time (T_h), and peak consumption ($1/T_h$) of unexposed or exposed females of the predatory mite *T. (A.) ornatus* preying upon adults of the coconut mite *A. guerreronis* for 24 h. Exposed predators had contact with LC₉₉ of degummed soybean and babassu oils, as estimated for *A. guerreronis*. Means followed by the same letter in the column do not differ by confidence interval at 5% probability level.

Exposure	$a \pm$ SE (95% CI)	$T_h \pm$ SE (95% CI)	$1/T_h \pm$ SE (95% CI)
Unexposed	0.0055 \pm 0.0010 a (0.0034–0.0075)	0.1036 \pm 0.0008 b (0.1019–0.1054)	9.65 \pm 1.16 b (9.48–9.81)
Degummed soybean oil	0.0080 \pm 0.0004 a (0.0004–0.0166)	0.0962 \pm 0.0014 c (0.0934–0.0990)	10.39 \pm 0.71 a (10.10–10.70)
Babassu oil	0.0063 \pm 0.0018 a (0.0026–0.0102)	0.1101 \pm 0.0014 a (0.1073–0.1130)	9.08 \pm 0.68 c (8.84–9.34)

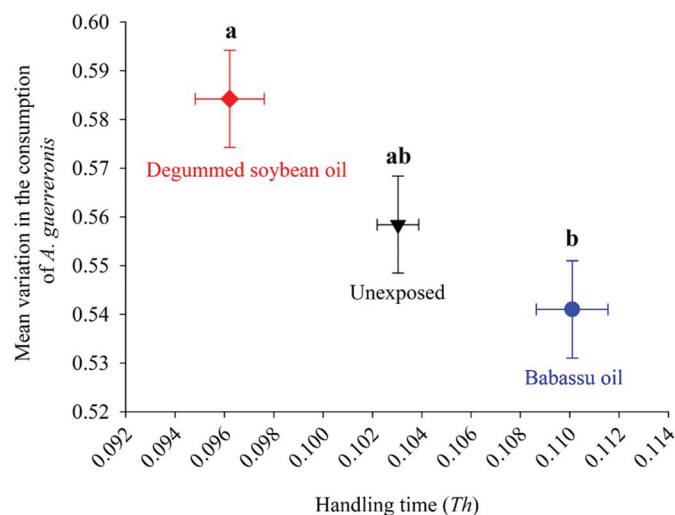


Figure 3. Variation in the consumption (Mean \pm SE) of the coconut mite *A. guerreronis* by females of the predatory mite *T. (A.) ornatus* unexposed or exposed to degummed soybean or babassu oils as a function of handling time (Mean \pm SE). Exposed predators had contact with LC₉₉ of degummed soybean and babassu oils, as estimated for *A. guerreronis*. Different lowercase letters indicate significant differences between treatments by Tukey HSD test ($P < 0.05$).

Discussion

In our study, regardless of the exposure to vegetable oils, *T. (A.) ornatus* showed a type III functional response when fed *A. guerreronis*. Additionally, Freitas et al. (2018) showed that females of *T. (A.) ornatus* exhibited type III functional response when fed *A. guerreronis* exposed and unexposed to coconut (*Cocos nucifera* L.) oil, indicating the non-influence of vegetable oil on the predation behaviour of this predator. Predators that exhibit type III functional response are important biological control agents (Fathipour and Maleknia 2016) and are effective in suppressing prey populations, especially at their high densities (Jumbo et al. 2019). Therefore, our results suggest that *T. (A.) ornatus* maintains its potential to prey on large populations of *A. guerreronis* even when exposed to the vegetable oils tested here.

In this study, regardless of exposure to vegetable oils, females of *T. (A.) ornatus* reached a limit of consumption at the density of 200 *A. guerreronis*, followed by a reduction in the proportion of prey consumed. This may be related to the satiety of the predator, because when it is reached, the consumption capacity decreases with the increase in offered prey (Ricklefs 2010) and this behaviour characterizes a type III functional response (Omkar 2005).

Together with the functional response, the parameters of attack rate (a) and handling time (T_h) help in determining the efficiency of the biological control agent (Holling 1959; Jumbo et al. 2019). The attack rate determines the capture capacity of a predator within a given area (Holling 1959), whereas handling time is characterized as the time spent by the predator to attack, kill, and eat a prey (Holling 1959). In this

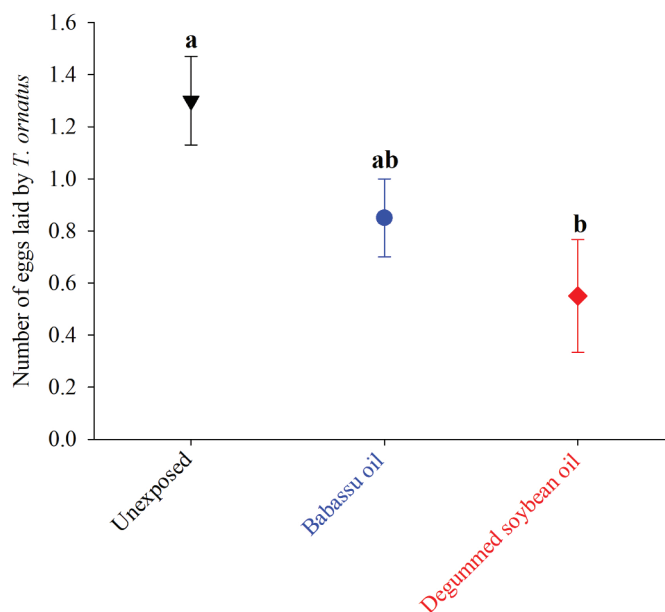


Figure 4. Oviposition (Mean \pm SE) of adult females of the predatory mite *T. (A.) ornatus* unexposed or exposed to degummed soybean or babassu oils at intermediate density (200 of *A. guerreronis*; $n = 10$ per treatment) counted after 24 and 48 h of spraying. Exposed predators had contact with LC₉₉ of degummed soybean and babassu oils, as estimated for *A. guerreronis*. Different lowercase letters in the columns denote statistical differences according to the Kruskal-Wallis nonparametric test ($P < 0.05$).

study, the attack rate of the predator was not altered by exposure to the evaluated vegetable oils. However, *T. (A.) ornatus* females exposed to babassu oil showed longer handling time and decreased peak consumption. Predatory mites can have their handling times prolonged when exposed to synthetic and botanical acaricides, decreasing their potential to capture preys and this decrease is the result of irritation and/or alteration of behavioural parameters (Lima et al. 2015; Teodoro et al. 2020). Although babassu oil is not considered repellent/irritant for *T. (A.) ornatus* (Saraiva et al. 2020), the presence of particular chemical compounds of this oil, especially lauric acid (26.16%) (Oliveira et al. 2017), may partially explain the results obtained in this study. The oxidation of lauric acid, usually, produces methyl ketones that are considered toxic to arthropods and can compromise the feeding of mites (Chatzivasilieiadis and Sabelis 1997; Antonious et al. 2003; Braga et al. 2007; Liu et al. 2015). Indeed, females of *Tetranychus urticae* Koch (Acari: Tetranychidae) avoided consuming leaf surfaces of common bean (*Phaseolus vulgaris*, cv. "Arena") treated with these substances (Chatzivasilieiadis and Sabelis 1997).

The handling time of *T. (A.) ornatus* when exposed to degummed soybean oil was shorter than that observed for babassu oil. This may explain the 12.6% increase in the peak consumption of *T. (A.) ornatus* in relation to exposure to babassu oil. It is reasonable to speculate that the presence of degummed soybean oil may have favoured the rates of an encounter between predator and prey and consequently the shorter time of prey handling. This possibility is supported by the variation in prey consumption in the treatment with degummed soybean oil, in which the shorter handling time of *T. (A.) ornatus* females was invested in the attack of other preys, leading to a greater variation in consumption when compared to babassu oil. Lima et al. (2015), in tests with azadirachtin on the predator *N. baraki*, also observed a large variation in the consumption of *A. guerreronis* due to a shorter handling time.

Despite consuming more preys, when exposed to degummed soybean oil, females of *T. (A.) ornatus* reduced the rate of oviposition. Although more detailed studies should be

conducted, this oil likely altered the behavioural parameters of the predator, because degummed soybean oil has a deterrent effect on oviposition in arthropods (Pacheco et al. 1995; Akter et al. 2019). In addition, linoleic acid found at higher concentration in degummed soybean oil (58.65%) compared to babassu (22.64%) (Oliveira et al. 2017) may have influenced this result, as it is a fatty acid that interferes with the oviposition of arthropods in a dose-dependent manner (Hwang et al. 1984; Don-Pedro 1990; Zhang et al. 2019). Our results suggest that degummed soybean oil may somehow interfere in the fertility of *T. (A.) ornatus* females, which in turn may influence the population growth of the predator with consequences on the biological control of *A. guerreronis*. Indeed, the exposure of *T. (A.) ornatus* to LC₉₉ of babassu and degummed soybean oils reduced the demographic rates of population growth of this phytoseiid, but a more pronounced reduction was found with degummed soybean oil (Saraiva et al. 2020). Additionally, similar results were found by Teodoro et al. (2020), in which the oviposition of *N. baraki* females decreased with exposure to degummed soybean oil.

Thus, we can conclude that babassu and degummed soybean oils are generally compatible with *T. (A.) ornatus* by not interfering in the predation on *A. guerreronis*, but the oviposition of the predatory mite may be reduced when subjected to degummed soybean oil, which emphasizes the necessity for further investigations about the factors that lead to such reduction in oviposition.

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Disclosure statement

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