

Effectiveness of three species of predatory mites (Acari: Phytoseiidae) for controlling *Raoiella indica* (Acari: Tenuipalpidae)



Eficiencia de tres especies de ácaros depredadores (Acari: Phytoseiidae) para el control de *Raoiella indica* (Acari: Tenuipalpidae)

<https://eqrcode.co/a/Q3XYz5>

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ABSTRACT: In this work, the efficiency of *Amblyseius largoensis* (Muma), *Typhlodromus (Anthoseius) ornatus* Denmark e Muma and *Iphiseiodes zuluagai* Denmark (Muma for controlling *Raoiella indica* Hirst (Acari: Tenuipalpidae) was assessed by comparing their functional and numerical responses to increasing densities of *R. indica* eggs. The three predatory mites exhibited a type II functional response, indicating that they can assist in the control of *R. indica*, especially at low to moderate densities of this pest. However, *A. largoensis* consumed the highest number of preys with the shortest handling time and showed a superior reproductive potential when fed upon *R. indica* eggs. *A. largoensis* proved to be the most efficient of the three predatory mites.

Keywords: biological control, IPM, phytoseiids, predation, red palm mite.

RESUMEN: En este trabajo se evaluó la eficiencia de *Amblyseius largoensis* (Muma), *Typhlodromus (Anthoseius) ornatus* Denmark (Muma e *Iphiseiodes zuluagai* Denmark & Muma para controlar *Raoiella indica* Hirst (Acari: Tenuipalpidae) mediante la comparación de sus respuestas numéricas y funcionales con densidades crecientes de huevos de *R. indica*. Los tres ácaros depredadores mostraron una respuesta de tipo II, lo que indica que pueden ayudar en el control de *R. indica*, especialmente en densidades de bajas a moderadas de esta plaga. Sin embargo, *A. largoensis* consumió el mayor número de presas en menor tiempo, así como presentó un potencial reproductivo superior cuando se alimentó de huevos de *R. indica*. *A. largoensis* demostró ser el más eficiente de los tres ácaros depredadores.

Palabras clave: ácaro rojo del cocotero, control biológico, depredación, fitoseidos, MIP.

INTRODUCTION

The red palm mite, *Raoiella indica* Hirst (Acari: Tenuipalpidae), is an invasive pest in the New World with high potential for dispersal and establishment in new areas (1). This mite can reach high population densities and inflict severe economic losses to ornamental plants, banana,

and several palm trees, especially coconut (*Cocos nucifera* L.) (2-5). In Brazil, the red palm mite was first reported in the northern state of Roraima in 2009 (6), and since then it has spread to other regions, including to the Northeast, the main coconut producing region in the country (7).

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Received: 30/07/2019

Accepted: 04/03/2020

Recent research has focused on biological (2, 8) and chemical control (9) strategies aiming at reducing the problems associated with infestation of *R. indica*. Although pesticides have shown to efficiently control *R. indica* (9), to date none of them is registered in Brazil to control this pest in coconut fields (10). Therefore, a growing concern is the use of broad-spectrum pesticides to control this pest.

Regarding biological control, several studies have been conducted to identify and evaluate the potential of native predators in the control of *R. indica* (2, 7, 8, 11,12,13,14). Among predatory mites, phytoseiids are the most important natural enemies associated with *R. indica* (2, 11,12,13). *Amblyseius largoensis* Muma has been found in association with *R. indica* in coconut plantations in several countries, including in Brazil and Cuba (11,12,13). This predator feeds on all stages of *R. indica* and can be efficient in controlling the red palm mite, mainly at low pest densities (7, 14). Furthermore, *A. largoensis* can reduce *R. indica* densities under greenhouse conditions (14). In Brazil, *Iphiseiodes zuluagai* Denmark and Muma is also associated with *R. indica* (11), whereas *Typhlodromus (Anthoseius) ornatus* Denmark and Muma is often found foraging on coconut trees (15, 16). Therefore, it is reasonable to conjecture that such predatory mites could act as biological control agent of this pest in the field.

One of the most important approaches to evaluate the potential of predators as biological control agents is the study of their functional and numerical responses (17, 18). The functional response assesses the predation rate in relation to prey density (19), whereas the numerical response evaluates the variation at the predator population density in response to changes in prey densities. Accordingly, the efficacy of *T. ornatus*, *A. largoensis*, and *I. zuluagai* was assessed by comparing their functional and numerical responses to increasing densities of *R. indica* eggs. We aimed to evaluate the potential of these three predatory mite species as biological control agents of *R. indica*.

MATERIAL AND METHODS

Rearing of predatory mites

Stock colonies of *A. largoensis* and *I. zuluagai* were established with individuals collected from unsprayed coconut leaf of the dwarf green variety in Aracaju (10° 54' 36" S, 37° 04' 12" W) and

Neópolis (10°19' 12" S, 36°34' 46" W) cities, respectively, Sergipe State, Brazil. Colonies of *T. ornatus* were initiated with individuals collected from unsprayed coconut fruits of the dwarf green variety in São Luís city (02° 35' 03" S, 44° 12' 32" W), Maranhão State, Brazil. Mite species identification was performed using taxonomic keys, and voucher specimens were deposited in the collection of Maranhão State University (UEMA), São Luís, Brazil.

Colonies of *T. ornatus* were maintained under controlled laboratory conditions (27 ± 3°C temperature, 70 ± 10 % relative humidity and a 12 h photoperiod) on rectangular PVC sheets (23 cm length x 4 cm width) sitting on water-soaked polyurethane foam (24 cm length x 5 cm width x 0.33cm depth) placed in a plastic tray. A barrier of water-soaked cotton wool (1 cm high) was placed around the edge of the PVC sheet to prevent the mites from escaping. Cotton threads under cover slips (18 x 18 mm) were placed on PVC sheets as shelter and oviposition sites. Pollen of castor bean, *Ricinus communis* L., all developmental stages of *R. indica*, and honey were provided every other day as a food source.

Functional and numerical responses

Bioassays were performed under the same environmental conditions used for rearing. Experimental units consisted of PVC discs (6 cm diameter) sitting on water-soaked polyurethane foam (6 cm diameter x 0.33 cm depth) inside a plastic container (6.2 cm diameter x 5 cm depth) without lid. A water-soaked cotton wool barrier (1 cm high) was placed around the edge of the PVC disc to confine mites.

Bioassays were conducted separately for each predatory mite species (i.e. *T. ornatus*, *A. largoensis*, *I. zuluagai*). In short, coconut leaflet sections (1cm²) containing 5, 10, 20, 30, 40, 50 and 80 eggs of *R. indica* were transferred to each PVC disc. Eggs of *R. indica* (1-3 day old) were taken from unsprayed coconut leaves. Subsequently, one mated female of each predator, at the beginning of its reproductive period (7-10 day old), was transferred to each disc containing increasing densities of *R. indica* eggs. Fourteen replicates were included for each egg density. To determine the functional response, the numbers

of prey killed were recorded after 24 hours without prey replacement. To assess the numerical response, the eggs laid by each predatory mite species in relation to prey density were evaluated during 2 days, with prey replacement at the end of the first day. Data of oviposition on the first day were discarded to minimize the effect of previous diets (7).

Statistical analyses

For each predatory mite species, the type of the functional response curve was estimated using a logistic regression analysis of the proportion of the prey killed in relation to prey density following the protocol of Juliano (20) using Proc CATMOD of SAS software (21). The linear coefficient sign of the equation generated from the proportion of prey killed in relation to the original density of prey was used to determine the type of functional response (19). The linear coefficient, if not significant, indicates a type I functional response (linear rise in prey consumption as a function of prey density); when significant and with a negative sign, it indicates a type II functional response (increase in prey consumption with prey density to a plateau - predator saturation); and when significant and with a positive sign, it denotes a type III functional response (accelerated rise in prey consumption with prey density rendering a sigmoidal curve). The functional response is based on the parameters handling time (Th), which involves the killing and ingestion of prey, and the attack rate (a'), which is the efficiency in prey searching (19, 22). These parameters were subsequently estimated using nonlinear regression with the method of least squares (PROC NLIN SAS) (21). As the experiments were conducted without prey replacement during the functional response experiment, the random predator equation (23) was used as a description of the type II functional response.

$$N_e = N_0\{1 - \exp[\alpha(T_h N_e - T)]\}$$

where N_e is the number of prey consumed, N_0 is the initial density of prey, T is the time interval (24 hours), α is the attack rate, T_h is the handling time. The consumption peak was calculated for each predatory mite based on the reciprocal of Th

$(\frac{1}{Th})(\frac{1}{Th})$ and compared using confidence intervals. The variation in prey consumption for each predator at each density was calculated according to the following equation:

$\Delta N_a = \frac{NaN_{max} - NaN_{min}}{N_{max} - N_{min}}$, where NaN_{min} and NaN_{max} stand for the minimum and maximum numbers of prey consumed, respectively. N_{min} and N_{max} are the minimum and maximum prey densities (24). The variation in prey consumption was subjected to one-way ANOVA followed by Tukey test using the software SAS (21). Oviposition rates of the three predatory mites as a function of *R. indica* egg density were submitted to a regression analyses using PROC REG of SAS Software (21).

RESULTS

The regression analyses generated significant linear coefficients with negative signs, indicating that *T. ornatus*, *A. largoensis*, and *I. zuluagai* presented type II functional responses to eggs of *R. indica* (Table 1). For all predator species, the number of prey consumed increased with egg density, (Fig 1). The predator *A. largoensis* consumed close to 100 % prey up to the density of 40 *R. indica* eggs. In contrast, *T. ornatus* and *I. zuluagai* consumed the same amount of prey up to the densities of 10 and 5 *R. indica* eggs, respectively (Fig 2). At highest prey density (80), *A. largoensis* consumed roughly 60 % of *R. indica* eggs, whereas *I. zuluagai* and *T. ornatus* killed 26 % and 32 %, respectively.

The attack rate (a') did not vary among predator species. Handling time (Th) was shorter for *A. largoensis* in comparison with *T. ornatus* and *I. zuluagai* (Table 2). The prey consumption peak estimated for *A. largoensis* was higher than those estimated for *T. ornatus* and *I. zuluagai* (Table 2). The predator *A. largoensis* also had a higher variation in the consumption of *R. indica* eggs in comparison with the remaining two species ($F_{2,36} = 66.36, p < 0.001$) (Fig 3).

The number of eggs laid by *A. largoensis* steadily increased with prey density, peaking at 50 *R. indica* eggs and decreasing afterwards (Fig 4) ($Y = -0.33149 + 0.008510x - 0.00081997x^2$, $r^2 = 0.98, P = 0.0003$). The oviposition of *T.*

ornatus linearly increased with prey density (Fig 4) ($y = 0.35528 + 0.01176x$, $r^2 = 0.87$, $P = 0.0022$ (Fig 4). Unlike *A. largoensis* and *T. ornatus*, the oviposition of *I. zuluagai* was not related to *R. indica* egg density ($p > 0.05$).

DISCUSSION

The predatory mites *A. largoensis*, *I. zuluagai* and *T. ornatus* exhibited a type II functional response to eggs of *R. indica*, in which there was an increase in consumption due to a greater

availability of prey up to a certain density, reaching stability at high densities (19), which may be associated with satiety of the predator. These results indicate that these predators are more efficient at low to moderate prey densities.

Phytoseiid mites usually present type II functional responses to pest mites (25). For instance, type II response curves were also observed for the phytoseiids *Typhlodromus pyri* Scheuten preying upon protonymphs and deutonymphs of the European red mite

Table 1. Estimated parameters of the logistic regression of the proportion of *Raoiella indica* eggs consumed by females of three species of predatory mites/ Parámetros estimados de la regresión logística de la proporción de huevos de *Raoiella indica* consumidos por hembras de tres especies de ácaros depredadores.

Species	Parameters	Value (\pm SE)	df	χ^2	P	Functional response
<i>T. ornatus</i>	Intercept	7.0937 \pm 0.8198	1	74.8	<0.0001	
	Linear	-0.3955 \pm 0.0604	1	42.8	<0.0001	
	Quadratic	0.00703 \pm 0.00136	1	26.7	<0.0001	II
	Cubic	-0.00004 \pm 9.184E-6	1	20.7	<0.0001	
<i>A. largoensis</i>	Intercept	27.9692 \pm 4.1455	1	45.5	<0.0001	
	Linear	-0.8248 \pm 0.1363	1	36.6	<0.0001	
	Quadratic	0.00599 \pm 0.00106	1	32.1	<0.0001	II
	Cubic	0.000108 \pm 0.000014	1	55.7	<0.0001	
<i>I. zuluagai</i>	Intercept	5.5486 \pm 0.6772	1	67.1	<0.0001	
	Linear	-0.2848 \pm 0.0515	1	30.6	<0.0001	II
	Quadratic	0.00482 \pm 0.00119	1	16.48	<0.0001	
	Cubic	-0.00003 \pm 8.158E-6	1	11.75	0.0006	

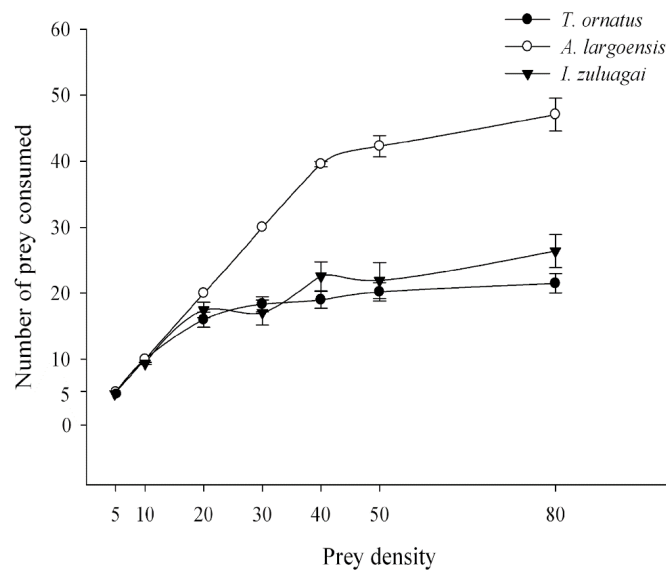


Fig. 1. Mean number (\pm SE) of *Raoiella indica* eggs consumed by females of three species of predatory mites in relation to prey density. /Número medio (\pm EE) de huevos de *Raoiella indica* consumidos por hembras de tres especies de ácaros depredadores con relación a la densidad de presas.

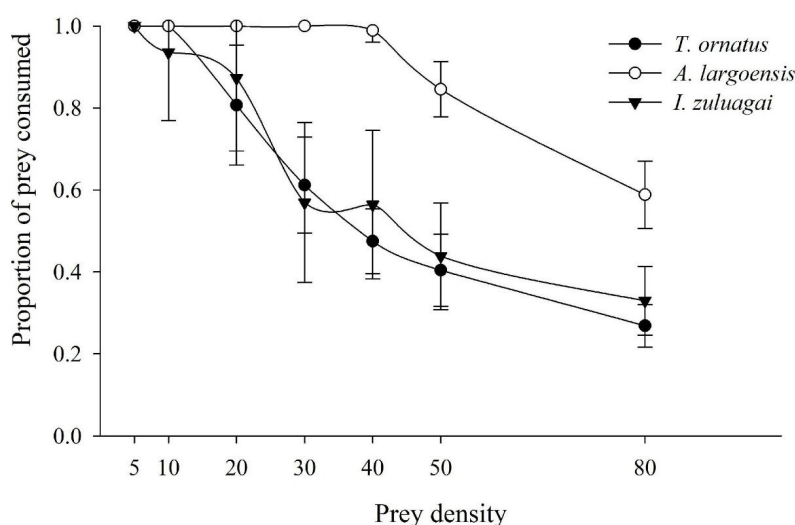


Fig. 2. Mean (\pm SE) proportion of *Raoiella indica* eggs consumed by females of three species of predatory mites in relation to prey density. / Proporción media (\pm EE) de huevos de *Raoiella indica* consumidos por hembras de tres especies de ácaros depredadores con relación a la densidad de presas.

Table 2. Estimates of the parameters attack rate (a'), handling time (Th) and consumption peak of three species of predatory mites preying upon eggs of *Raoiella indica* for 24 hours/ Estimaciones de los parámetros tasa de ataque (a'), tiempo de manipulación (Th) y pico de consumo de tres especies de ácaros depredadores depredando huevos de *Raoiella indica* por 24 horas.

	$a' \pm SE$ (95% CI)	$Th \pm SE$ (95% CI)	$\left(\frac{1}{Th}\right)\left(\frac{1}{Th}\right)$ (95% CI)
<i>T. ornatus</i>	0.0101 ^a \pm 0.0013 (0.0074 - 0.0126)	1.1234 ^b \pm 0.0301 (1.0636 - 1.1832)	0.981 ^b (0.845 - 0.941)
<i>A. largoensis</i>	0.0205 ^a \pm 0.0047 (0.0111 - 0.0299)	0.5064 ^a \pm 0.0094 (0.4877 - 0.5252)	1.975 ^a (1.904 - 2.049)
<i>I. zuluagai</i>	0.0108 ^a \pm 0.0033 (0.0040 - 0.0176)	0.9924 ^b \pm 0.0442 (0.9045 - 1.0803)	1.00 ^b (0.930 - 1.111)

Means followed by same letter within a column do not differ based on confidence intervals. / Las medias seguidas por la misma letra dentro de una columna no difieren según los intervalos de confianza.

Panonychus ulmi Koch (Acari: Tetranychidae) (26), *Euseius alatus* DeLeon, and *Amblyseius herbicolus* (Chant) feeding on larvae and nymphs of the false spider mite *Brevipalpus phoenicis* Geijkes (Acari: Tenuipalpidae) (27, 28). In agreement with our results, Carrillo & Peña (7) and Mendes *et al* (29) also found this type of functional response in *A. largoensis* feeding upon eggs of *R. indica*.

The phytoseiids *A. largoensis*, *I. zuluagai*, and *T. ornatus* are classified as generalist type III predatory mites. They feed upon pest mites and small arthropods as well as pollen and sugary

exudates (30), which helps to sustain their populations even during scarcity. Due to their feeding habits, the populations of generalist predators tend to disperse less to new patches and are more stable than specialist predator populations in agroecosystems (31). The consumption curves were similar among predators, and only the average amount of eggs preyed by them varied. *A. largoensis* was more efficient, consuming around 1.5 to 2 times more eggs at the highest prey density than *I. zuluagai* and *T. ornatus*, respectively. This differential consumption among predators could be explained

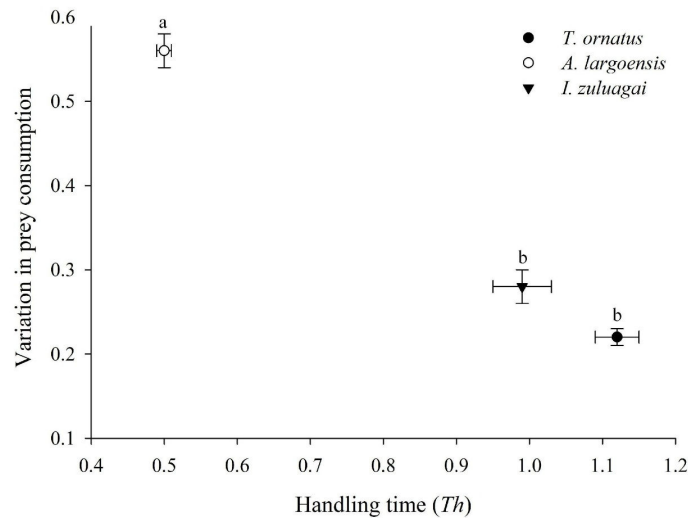


Fig. 3. Mean (\pm SE) variation in *Raoiella indica* eggs consumption by females of three species of predatory mites in relation to handling time. Equal letters do not differ significantly by Tukey tests. / Variación media (\pm EE) en el consumo de huevos de *Raoiella indica* por hembras de tres especies de ácaros depredadores con relación al tiempo de manipulación. Las letras iguales no difieren significativamente según las pruebas de Tukey.

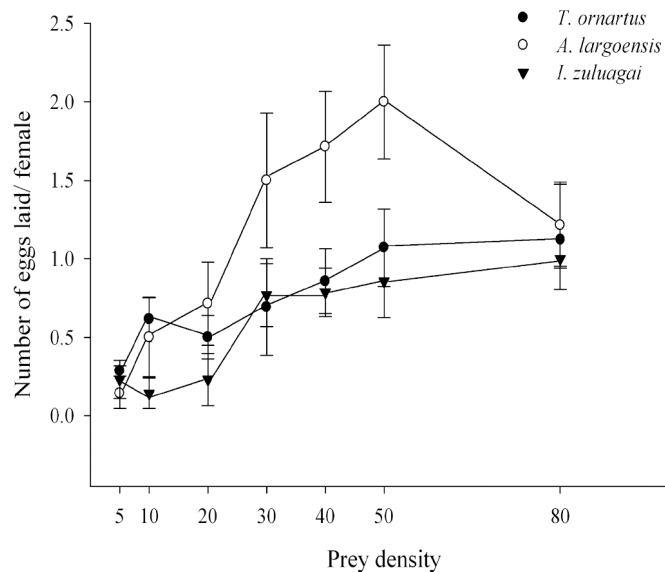


Fig. 4. Mean (\pm SE) number of eggs laid by females of three species of predatory mites in relation to *Raoiella indica* egg density. / Número medio de huevos (\pm EE) depositados por hembras de tres especies de ácaros depredadores con relación a la densidad de huevos de *Raoiella indica*.

by the relative size of each predator species (32), since *A. largoensis* and *I. zuluagai* are larger than *T. ornatus*. Furthermore, *A. largoensis* and *I. zuluagai* are more active than *T. ornatus*, a behavior that may increase the probability of finding prey (18), as well as increasing the

energy expenditure of predators leading to increasing prey consumption.

The proportion of prey consumed by *A. largoensis* was close to 1 up to 40 *R. indica* eggs, in line with Carrillo & Peña (7). In contrast, the proportion of prey consumed by *I. zuluagai* and *T. ornatus* was close to 1 only at the lowest

densities (5 and 10 *R. indica* eggs, respectively), probably due to the difficulty of both predators in finding prey at low densities (26).

Carrillo & Peña (7) showed that *A. largoensis* significantly preferred and consumed more eggs of *R. indica* than its immatures stages or adults. Eggs of *R. indica* are easily accessible for predatory mites because they last for approximately 9 days, the longest developmental stage of this pest (33). In addition, *R. indica* eggs do not exhibit antipredator behavior. Here, the attack rate (a') did not vary among predators; however, *A. largoensis* consumed more *R. indica* eggs in shorter time compared to *I. zuluagai* and *T. ornatus*. According to Holling (19), the handling time includes the period necessary to kill and consume the prey. A longer handling time may suggest that the predator spends a longer period with one prey, taking a longer time to find and consume another prey (34). Therefore, *A. largoensis* needs less time to consume *R. indica* eggs, which may result in more time to attack and catch another prey. This can be observed by the negative relationship between variation in prey consumption and handling time, in which *A. largoensis* had a shorter manipulation period and a higher consumption variation when compared to *T. ornatus* and *I. zuluagai* (Fig 3). This relationship can also be altered in conspecific populations that have different times of association with the pest; for instance, native populations of *A. largoensis* in long association with *R. indica* exhibit a more aggressive behavior, a greater variation in prey consumption, and, consequently, a shorter handling time than those populations that have not been in contact with the pest (29).

The number of eggs laid by *A. largoensis* and *T. ornatus* females increased with prey density, indicating that these predators obtained nutritional benefits that promoted reproduction (35). This may indicate that the consumption of *R. indica* eggs by these predators may contribute to their numerical increase in the field. Similarly, females of *Euseius concordis* Chant (Acari: Phytoseiidae) oviposited more when fed upon eggs of the cassava green mite *Mononychellus tanajoa* Bondar (Acari: Tetranychidae) than when they did on immature stages or adults (35). Furthermore, *A. largoensis* preferred eggs over

other developmental stages of *R. indica* and also exhibited a similar oviposition peak (7). In contrast, *I. zuluagai* oviposition was not related to prey density, suggesting that *R. indica* eggs are not an optimal developmental stage for its reproduction. However, it is possible that the consumption of mixed-life stages or other developmental stages of *R. indica* are more suitable for *I. zuluagai* as observed for other phytoseiid predators. For instance, *P. persimilis*, *G. occidentalis*, and *N. californicus* preferred nymphs to eggs of *P. citri*, suggesting that consumption of nymphs was more profitable in terms of nutritional value for these predators (18).

Our results indicate that the predatory mites *A. largoensis*, *I. zuluagai*, and *T. ornatus* may contribute to the control of *R. indica*, mainly at low to moderate densities. However, *A. largoensis* was the most efficient predator because it consumed the greatest number of prey with the shortest handling time and showed the highest reproductive potential when fed upon *R. indica*. Further field studies are needed to confirm the potential of these phytoseiids in controlling *R. indica*, especially *A. largoensis*. However, as these three predatory mites co-occur on coconut palms, further research should also focus on whether they could have an additive, neutral or negative effect on *R. indica* control in the field. Intraguild interactions among these predators could play a role in mediating biological control of *R. indica* when these predators are used. Also, augmentation or mass field releases should be evaluated as strategies for *R. indica* management.

ACKNOWLEDGEMENTS

Funding was provided by the National Council for Scientific and Technological Development (CNPq) and the Maranhão State Foundation for Research Aid (FAPEMA).

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Los autores declaran que no existe conflicto de intereses

Author Contribution Statement: **Caroline Rabelo Coelho:** Desarrolló los prototipos y realizó pruebas para evaluar los resultados. Participó en la búsqueda de información y en el análisis y en la revisión y redacción del informe final. **Andreia Serra Galvão:** Participó en la búsqueda de información, en el diseño de la investigación. Participó en el análisis de los resultados y redacción del borrador del artículo y la revisión crítica de su contenido y en la aprobación final. **Maria Clezia dos Santos:** Participó en las pruebas realizadas para evaluar los resultados. Participó en la recolección de los datos. Participó en la revisión crítica de su contenido y en la aprobación final. **Adriano Pimentel Farias:** Colaboró en la investigación, contribuyó al desarrollo de los ensayos, prototipos y participó en las pruebas realizadas para evaluar los resultados. Participó en la revisión crítica de su contenido y en la aprobación final. **Adenir Vieira Teodoro:** Concibió la idea; Colaboró en la investigación, contribuyó al desarrollo de los prototipos. Participó en la búsqueda de información. Participó en la redacción del borrador del artículo y la revisión crítica de su contenido y en la aprobación final.

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