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Ethanolic extracts of *Brosimum alicastrum* and *Plectranthus amboinicus* for the control of *Raoiella indica*

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Abstract
The acaricidal and repellent activity of three ethanolic extracts of two plant species on *Raoiella indica* was evaluated under laboratory conditions. The ethanolic extract of *Plectranthus amboinicus* caused the highest repellence of 61% over *R. indica* at 96 hours of evaluation, being statistically superior to the other extracts. Extracts of leaves and seeds of *Brosimum alicastrum* caused maximum 24% repellency. It was also observed that three extracts can not be considered as potential acaricides, due to the low mortality that they caused on *R. indica*.

Keywords: Mite, plant extracts, acaricidal activity, biological control

Introduction
The mite of the palms (*Raoiella indica* Hirst), is a quarantine important pest that damages coconut trees, palm trees, as well as bananas in different parts of the world [1]. The mite is a polyphagous species that reach high populations and causes negative impacts in islands of Caribbean, Florida, northern of South America and Mexico [2]. In Mexico it was declared officially present on November 20, 2009 in the state of Quintana Roo and phytosanitary actions have been implemented with the objective of reducing the infestation levels of *R. indica* in coconut palms, other species of palm and banana [3].
The combat of *R. indica* is carried out through the periodic application of acaricides and the elimination of the infested plant material (sanitary pruning). Among the products that have shown better effectiveness in the control of said pest are abamectin, spiridiclofen and sulfur [4]. Peña *et al*. (2007) [5] found that the applications of spiromesifen, dicofol and acequinocyl in coconut plantations were effective for the reduction of populations of the *R. indica* in Puerto Rico, while in Florida this mite was controlled with aspersions of etoxazole, abamectin, pyridaben, milbemectin and sulfur. However, Feiber (2009) [6] found in Florida, that control measures in nurseries have been impractical, because the infestation pressure of palm trees by *R. indica* is strong, which makes the use of acaricides unviable to have a 100% control.
Given the difficulty of controlling this mite by the use of conventional chemicals, the application of plant extracts could be an important alternative; since the use does not consider total eradication, but seeks the reduction of populations, seeking to restore the biological balance [7].
For this reason the present work aims to evaluate the acaricidal activity of *Brosimum alicastrum* Swartz and *Plectranthus amboinicus* Sprengel extracts as a sustainable alternative for the control of *R. indica*.

Materials and methods
Vegetal material
The leaves and seeds of *B. alicastrum* and leaves of *P. amboinicus* were collected from plants located in the city of Tizimin, Yucatan. The collection was made when they reached physiological maturity. The sampling was systematic collecting the leaves and fruits that were sufficient for the investigation. The study time was five months between august and december of 2016.
The plant material was taken to the laboratory where they were washed with distilled water to eliminate any foreign matter that was present in the case of the leaves, in the case of the fruits

of *B. alicastrum* these were pulped and dried at room temperature for three days, later, they were submitted in a forced air oven for 48 h at 60 °C.

After being completely dry, the leaves and seeds were ground with a willy® mill and the ground material was stored in a glass container for storage at room temperature.

Ethanolic extraction

To perform the ethanolic extraction, 5 (leaves) and 10 (seed) g of the ground material were weighed on an ADAM model PGW 3502i® scale, which was introduced into an amber bottle and 266 ml of ethanol was added, this mixture was put a constant stirring of 650 (rpm) on a stirring hot plate CORNING PC-420D® for one week at room temperature, every 48 hours of agitation the ethanol was filtered with 15 cm Whatman filter paper and again 266 ml of ethanol was added; this process was repeated three times until the end of the week, the filtered ethanol was kept refrigerated (a total of 750 ml was recovered).

The ethanolic extraction was vaporized in a distiller until about 90% of ethanol (675 ml) was recovered; The final product is the extract that was used in the bioassays.

Mite collection

It was carried out in the Research Area of the Technological Institute of Tizimin, coconut plants were identified that presented the symptomatology caused by *R. indica*, such as yellowing, drying and death of the leaves; once identified, the underside of the leaves was observed with a hand magnifying glass to verify the presence of *R. indica*. Finally, the leaves were detached where greater conglomeration was observed, subsequently it was deposited in a previously labeled container with date, time and place of collection. The leaves were then taken to the parasitology laboratory for identification using a Optika Microscopes Italy® brand stereoscope, where characteristics similar to those described in literature were observed and corroboration of the presence of *R. indica*.

Bioassays of mortality

In petri dishes of 9 cm in diameter, a piece of coconut palm leaf (2.5 x 4.5 cm) was placed, which served as food for *R. indica*. The leaf was placed with the back facing up and at each end was inserted Oasis® floral sponge saturated with distilled water to keep the leaves hydrated.

In each petri dish were placed 20 adult mites, which were manipulated with the help of a stereoscope and entomological pins, for each treatment were made 5 repetitions to give a total of 100 mites per treatment, which were kept at a constant temperature of 23 ± 2 °C.

Finally, the concentrations of the extracts of *B. alicastrum* and *P. amboinicus* were prepared for each treatment in an aqueous solution, in total there were 10 concentrations (0.3%, 0.5%, 0.8%, 1%, 2%, 3%, 5%, 7%, 10%, 13%) in relation to w/v, i.e. the concentration of 10%, one ml of extract was put in nine ml of distilled water, to do the above, a 10 ml Kimax® flask was used and a three ml pipette. Two treatments were prepared as control, ethanol and distilled water. All the concentrations of extract, ethanol and distilled water were applied to the treatments by the spray method with an atomizer.

The petri dishes were monitored at 24, 48, 72 and 96 h with a stereoscope and entomological pins, to perform the count of dead mites in each treatment.

Experimental design and statistical analysis

The experimental design was completely randomized in the bioassays, with five repetitions for each treatment. To evaluate the effect of the three extracts on the mortality and repellency of *R. indica*, it was carried out through a mixed effects model where the type and concentration of the extracts with interaction were evaluated as main factors, while the interval of time (24, 48, 72 and 96 h) and the replicas nested in this factor was considered as a random effect. The effect of the concentration for each species separately, with the same condition of the most complex model, was also evaluated using a mixed model using the function lme in the package nlme in the software R. In addition, a Probit analysis was performed to evaluate the LC50 ($p < 0.05$) using the function drc and dose in package drc and MASS also in Software R [8, -11].

Results

Significant effects of extract type and concentration were found separately on corrected mortality (Table 1). Of the three evaluated extracts, the leaf extract of *P. amboinicus* showed better results, the concentration of 13% was the highest effect, killing 13% of the adult individuals of *R. indica*. The best concentrations of the leaf and seed extracts of *B. alicastrum* did not kill more than 5% of the adult individuals of *R. indica* (Table 2).

In the repellency percentage, significant effects of the extract type and the concentration were found separately and in interaction (Table 1). The leaf of *P. amboinicus* presents the highest percentages of repellency in comparison with the leaf and seed extracts of *B. alicastrum* (Table 3). For both mortality (13%) and repellency (61%) the leaf extract of *P. amboinicus* presents the highest values at the highest concentration (13%).

Table 1: Results of the mixed models where the type and concentration of the extracts with interaction are evaluated as main factors, while the Time interval (24, 48, 72, 96 h) and the replicas nested in this factor were considered as a random effect. P value (<0.05 in bold).

Corrected Mortality				
Source	**All extracts**	**Seed *B. alicastrum***	**Leave *B. alicastrum***	**Leave *P. amboinicus***
Type of extract	$F_{2,20}$=235.5	-	-	-
Concentration	$F_{1,20}$= 36.6	$F_{1,20}$=40.43	$F_{1,20}$=61.34	$F_{1,20}$=12.23
Type of extract: Concentration	$F_{2,20}$= 2.1	-	-	-
	Repelence percentage			
	All extracts	Seed *B.alicastrum*	Leave *B. alicastrum*	Leave *P. amboinicus*
Type of extract	$F_{2,105}$=183.63	-	-	-
Concentration	$F_{11,105}$=14.88	$F_{11,33}$=47.64	$F_{11.33}$=5.88	$F_{11,33}$=27.42
Type of extract: Concentration	$F_{22,105}$= 10.86	-	-	-

Table 2: Average Corrected Mortality for each type of extract, EE by concentration level (Conc).

Conc	Seed *B. alicastrum*	EE±	Leave *B. alicastrum*	EE±	Leave *P. amboinicus*	EE±
0.3	1.55a	0.35	3.00a	0.30	8.90a	0.80
0.5	1.10a	0.25	2.80a	0.26	7.95a	0.69
0.8	1.45a	0.32	2.15b	0.30	4.10b	0.34
1	2.55a	0.57	4.40a	0.27	5.20b	0.80
2	2.15a	0.48	3.25a	0.10	7.30a	0.79
3	3.20a	0.72	3.35a	0.22	8.60a	0.82
5	4.15b	0.93	3.55a	0.17	11.15a	1.44
7	3.55a	0.79	4.20b	0.31	4.30b	0.49
10	1.90a	0.42	4.95b	0.32	5.70b	0.47
13	4.15b	0.93	3.95b	0.32	12.25a	1.05

Table 3: Percentage of average repellency for each type of extract, EE per level of concentration (Conc).

Conc	Seed *B. alicastrum*	EE ±	Leave *B. alicastrum*	EE ±	Leave *P. amboinicus*	EE ±
0.003	7.75a	2.45	14.50a	4.59	44.50a	14.07
0.005	5.50a	1.74	13.50a	4.27	39.25a	12.41
0.008	7.25a	2.29	9.50b	3.00	20.50a	6.48
0.01	12.25a	3.87	21.50c	6.80	25.25a	7.98
0.02	10.75a	3.40	15.50a	4.90	36.50a	11.54
0.03	16.00a	5.06	16.25a	5.14	42.75a	13.52
0.05	20.75b	6.56	17.50a	5.53	57.00b	18.02
0.07	18.00b	5.69	21.50c	6.80	21.50a	6.80
0.1	9.50a	3.00	25.00c	7.91	28.50a	9.01
0.13	20.75b	6.56	19.00c	6.01	60.75b	19.21
TA	4.25a	1.34	24.00c	7.59	12.75a	4.03
TE	9.75a	3.08	22.00c	6.96	15.25a	4.82

Discussion
In the present study, the extract from the leaves of *P. amboinicus* caused significant mortality and repellency against *R. indica* adults, this being the first report of acaricidal effect and repellency on *R. indica*.

It is recorded that the secondary metabolites of *P. amboinicus* allow it to present antioxidant, antibacterial [12], antitumor, cicatrizing, antiepileptic [13], larvicidal [14], anti-inflammatory, analgesic [15], insecticidal, repellent activity and acaricide [16]. In fact, Hegab *et al*. (2016) [16] evaluated *P. amboinicus* in two ways: 1) aqueous extract (dose of 30 ml/1 L of water) and 2) intercalated plants of *Cucumis sativus* with *P. amboinicus*, and observed that the intercalated cultures of *P. amboinicus* were effective in the control of *Bemisia tabaci*, *Thrips tabaci*; as well as in eggs and mobile stages of *Tetranychus urticae*; the aqueous extract was less effective.

In the present work *P. amboinicus* with the highest ethanolic extract concentration (13%) eliminated more than 10% of the individuals of *R. indica* exposed and the leaf and seed extracts of *B. alicastrum*, did not kill more than 5% of adult individuals of *R. indica*. According to these results none of the concentrations are considered suitable to be tested on palms or coconut trees, since, according to the criteria of Lagunes (1994) [17], which suggests that extracts that have more than 40% effectiveness on evaluated organisms are potential to work in open environments.

The extracts of leaf and seed of *B. alicastrum* do not present promising results in the present study, it is very probable that the secondary metabolites do not have acaricidal activity; there are studies that indicate that *B. alicastrum* has high phenolic content and has strong antioxidant activity of iron reduction compared to walnuts, almonds and peanuts [18].

It is recommended to carry out more studies that validate these effects and to test other forms of extraction, for example in *P. amboinicus* obtain the essential oils, since they present different biological activities [13].

Also, it would be convenient to test at the field level, the concentrations that had a high repellency effect. At the same time, possible allelopathic effects on palms or coconut should be evaluated, since it is mentioned that *P. amboinicus* present allelopathic effects [19].

Conclusion
The extracts used in this experiment show acaricidal and repellency activity on *R. indica*. The leaves of *P. amboinicus* cause significant mortality and repellency against adults of *R. indica*. Additional studies are needed to identify the active compounds responsible for its repellent and acaricidal activity. The highest concentration of *P. amboinicus* could be tested as a repellent in similar environmental conditions of *R. indica* to have better results and decide to consider it as an alternative in the sustainable protection of coconut and palm trees.

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References
1. Rodrigues JCV, Ochoa R, Kane EC. First report of *Raoiella indica* Hirst (Acari: Tenui- palpidae) and its damage to coconut palms in Puerto Rico and Culebra Island. International Journal of Acarology. 2007; 33:3-5.
2. Peña JE, Rodrigues JCV, Roda A, Carrillo D, Osborne LS. Predator-prey dynamics and strategies for control of the red palm mite (*Raoiella indica*) (Acari: Tenuipalpidae) in areas of invasion in the Neotropics. Proc. 2nd Meeting IOBC/ WPRS, Work Group Integrated Control of Plant Feeding Mites. Florence, Italy. 2009, 69-79.
3. Estrada-Venegas EG, Acuña-Soto JE, Chaires-Grijalva MP, Equihua-Martínez A. *Raoiella indica* Hisrt (Acari: Tenuipalpidae) su situación actual en el estado de Quintana roo, a ocho meses de su detección oficial. Folia

Entomológica Mexicana. 2015; 1:7-14.
4. SAGARPA. Ficha Técnica No. 14 Ácaro rojo de las palmas (*Raoiella indica* Hirst), 2014, 22.
5. Peña JE, Mannion CM, Osborne LS. Chemical Control of Red Palm Mite, *Raoiella indica*, on Ornamentals. In: guide to management of Red Palm Mite (RPM). University of Florida, 2007, 2.
6. Feiber D. Effectively managing red palm mites in nursery environments. Florida Department of Agriculture and Consumer Services, 2009, 2.
7. Gómez J, Hernández Y, Cabrera Y. Efecto acaricida de los extractos acuosos de plantas sobre el ácaro rojo (*Tetranychus tumidus* Banks). Agricultura Orgánica. 2010; 2:35-36.
8. Team RC. R: A language and environment for statistical computing, 2017.
9. Pinheiro J, Bates D, DebRoy S, Sarkar D, Team RC. Linear and Nonlinear Mixed Effects Models. R package version; 2007; 57:1-89. Version 3.1-137
10. Ritz C, Baty F, Streibig JC, Gerhard D. Dose-Response Analysis Using R Plos One. 2015; 10(12):e0146021.
11. Venables WN, Ripley BD. Modern Applied Statistics with S. Fourth Edition. Springer, New York. 2002, 140
12. Bhatt P, Negi PS. Antioxidant and antibacterial activities in the leaf extracts of Indian borage (*Plectranthus amboinicus*). Food and Nutrition Sciences. 2012; 3(02):146-152.
13. Arumugam G, Swamy MK, Sinniah UR. *Plectranthus amboinicus* (Lour.) Spreng: botanical, phytochemical, pharmacological and nutritional significance. Molecules. 2016; 21(4):369-394.
14. Jayaraman M, Senthilkumar A, Venkatesalu V. Evaluation of some aromatic plant extracts for mosquito larvicidal potential against *Culex quinquefasciatus*, *Aedes aegypti*, and *Anopheles stephensi*. Parasitology Research. 2015; 114(4):1511-1518.
15. Chiu YJ, Huang TH, Chiu CS, Lu TC, Chen YW, Peng WH, Chen CY. Analgesic and antiinflammatory activities of the aqueous extract from *Plectranthus amboinicus* (Lour.) Spreng. both *in vitro* and *in vivo*. Evidence-Based Complementary and Alternative Medicine. 2012; 2012:1-11.
16. Hegab MFA, Ayoub FH, Badran AB, Ammar MI. New approaches to control cucumber infestation with insects and mites with emphasis on the production and horticulture characteristics under greenhouse conditions. Annals of Agricultural Science Moshtohor. 2016; 54:629-638.
17. Lagunes A. Extractos, polvos vegetales y minerales para el combate de plagas del maíz y del frijol en la agricultura de subsistencia. Memoria. Colegio de Postgraduados en Ciencias Agrícolas, Montecillo, Texcoco, México, 1994, 35.
18. Ozer HK. Phenolic compositions and antioxidant activities of Maya nut (*Brosimum alicastrum*): Comparison with commercial nuts. International Journal of Food Properties. 2017; 20(11):2772-2781.
19. Pinheiro PF, Costa AV, Alves TDA, Galter IN, Pinheiro CA, Pereira AF *et al*. Phytotoxicity and cytotoxicity of essential oil from leaves of *Plectranthus amboinicus*, carvacrol, and thymol in plant bioassays. Journal of Agricultural and Food Chemistry. 2015; 63(41):8981-8990.