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Toxicity of acaricides to *Raoiella indica* and their selectivity for its predator, *Amblyseius largoensis* (Acari: Tenuipalpidae: Phytoseiidae)

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Abstract *Raoiella indica* Hirst (Acari: Tenuipalpidae) is considered a pest of coconut palm in Asia and the Middle East. This mite was recently introduced in the Americas, where it spread to several countries and expanded its range of hosts, causing heavy losses to coconut and banana production. The phytoseiid mite *Amblyseius largoensis* (Muma) is one of the predators most often encountered in coconut palms. Because the current prospects for the control of *R. indica* in the New World indicate the use of acaricides and the management of their natural enemies, the objective of this study was to evaluate the toxicity of selected acaricides to *R. indica* and the selectivity (i.e., toxicity to the predator relative to toxicity to the prey) for *A. largoensis*. Assays were performed by the immersion of banana leaf discs in acaricide solutions, followed by the placing of adult females of the pest or predator on the discs. Mortality of the mites was evaluated after 24 h, and the data obtained were subjected to probit analysis. Abamectin, fenpyroximate, milbemectin and spirodiclofen were the products most toxic to *R. indica* adults, whereas fenpyroximate and spirodiclofen were the most selective for *A. largoensis*.

Keywords Palm tree · Banana tree · Phytoseiid · Chemical control · Integrated management

Introduction

The red palm mite, *Raoiella indica* Hirst (Tenuipalpidae), was first described in India (Hirst 1924). Since then, this mite has been reported in several countries in Asia, the Middle East and Africa (Carrillo et al. 2012). Roughly 8 years ago, this mite was found on

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the French island of Martinique (Flechtmann and Etienne 2004) from which it rapidly spread to other Caribbean islands, Brazil, Colombia, USA, Mexico and Venezuela (Kane et al. 2005; Navia et al. 2011; Carrillo et al. 2012). In the Old World, *R. indica* has been found only on Arecaceae; however, in the New World, its range of hosts increased significantly, and it has been found developing and reproducing on species of Cannaceae, Heliconiaceae, Musaceae, Pandanaceae, Strelitziaceae and Zingiberaceae (Cocco and Hoy 2009; Navia et al. 2011; Carrillo et al. 2012). However, high populations in the New World have been observed only on palm and banana trees, where they cause a severe yellowing of the leaves followed by tissue necrosis (Flechtmann and Etienne 2004). Severe attacks of this mite on coconut palms (*Cocos nucifera* L.) have caused significant reductions in fruit production (Navia et al. 2011).

The control of *R. indica* has been investigated in areas where it has recently been introduced. Such practices as plant resistance (Rodrigues and Irish 2012), chemical control (Rodrigues and Peña 2012) and biological control (Peña et al. 2009; Carrillo et al. 2010, 2012; Carrillo and Peña 2012; Hoy 2012) have been previously explored. Investigations on the potential of natural enemies of *R. indica* have also been performed, particularly with *Amblyseius largoensis* (Muma) (Acari: Phytoseiidae) (Peña et al. 2009; Carrillo et al. 2010, 2012; Carrillo and Peña 2012).

Much information on the effectiveness of acaricides in the control of *R. indica* was obtained from field studies in India and the Middle East (Sarkar and Somchoudhury 1988; Jalaluddin and Mohanasundaram 1990; Jayaraj et al. 1991). However, the majority of the acaricides evaluated in those studies referred to products that are no longer allowed to be used in Brazil. In this country, the distribution of *R. indica* is restricted to the northern region, in the states of Roraima (Navia et al. 2011) and Amazonas (Rodrigues and Antony 2011). Although the Amazon rainforest predominates in these states, the crops of banana and both exotic and native Arecaceae, for example, açaí, moriche palm (buriti) and peach-palm, play important economic and social roles, particularly for low-income populations. The problems that *R. indica* may cause in this region should be considered along with its possible spread to the Brazilian northeast, where the majority of coconut palms in Brazil are located. A much more significant impact may occur in the northeast region with potentially serious consequences.

Because investigations on the potential of native and exotic natural enemies in Brazil have not reached any conclusive results, it is necessary to obtain information regarding the effectiveness of other control methods, permitting their use in emergency situations. The objective of this study was to evaluate the toxicity of selected acaricides to *R. indica* and the selectivity for *A. largoensis* under laboratory conditions.

Materials and methods

Collection and maintenance of *Raoiella indica* and *Amblyseius largoensis* populations

The specimens of *R. indica* used in this study were collected from colonies that were initiated with mites collected from *Adonidia merrillii* (Becc.) Becc. (=*Veitchia*) (Arecaceae) in Boa Vista, Roraima, Brazil (2°45′29.1″N, 60°43′51.6″W). The colonies were established into plastic dishes measuring 16 cm in diameter containing a banana leaf disc on a piece of polyethylene foam measuring 1.0 cm in thickness. The border of each disc was covered with a layer of hydrophilic cotton, and the foam and cotton were kept saturated with distilled water to prevent the escape of the mites. These units were

maintained inside growth chamber at 27 ± 0.5 °C, 80 ± 10 % relative humidity and a 12 h photoperiod. The specimens of *A. largoensis* used in the study were collected in the same location from coconut palm leaves and transferred to dishes infested with *R. indica*, as previously described. Castor bean (*Ricinus communis* L.) pollen was also added to feed the predator mites.

Acaricides evaluated

The acaricides evaluated were as follows: abamectin (Kraft 36 EC; Cheminova Brasil); chlorfenapyr (Pirate; Basf); diafenthiuron (Polo 500 WP; Syngenta Proteção de Cultivo); fenbutatin oxide (Torque 500 SC; Basf); fenpyroximate (Ortus 50 SC; Arysta Lifescience do Brasil Indústria Química e Agropecuária); hexythiazox (Talento; Du Pont do Brasil—Barueri); milbemectin (Milbeknock; Iharabras Chemical Industries); propargite (Omite 720 EC; Chemtura Indústria Química do Brasil); spirodiclofen (Envidor; Bayer, São Paulo, SP) and spiromesifen (Oberon 240 SC; Bayer CropScience).

Experimental procedure

Preliminary tests

Preliminary, tests were performed according to method No. 4 of the series of methods for susceptibility testing of the Insecticide Resistance Action Committee (IRAC 2003). Concentrations diluted by a factor of 10 (0.01, 0.1, 1, 10, 100 and 1,000 mg of active ingredient per liter of solution) and a control (distilled water) were prepared for each acaricide. Each experimental unit corresponded to a banana leaf disc (5 cm in diameter) immersed for 5 s in one of the acaricide solution or water. The disc was allowed to dry at room temperature for 20 min and was placed into a Petri dish (9 cm in diameter) containing a piece of polyethylene foam covered with a filter paper. The border of each disc was covered with hydrophilic cotton to prevent the mites escaping, and the foam was kept wet by the addition of distilled water. Ten adult females of *R. indica* or five adult females of *A. largoensis* were transferred to each leaf disc, representing one false repetition. Each treatment had 3 replications per concentration for a total of 30 and 15 mites, respectively. The units housing the mites were maintained inside growth chamber at 27 ± 0.5 °C, 80 ± 10 % RH and a 12 h photoperiod. The total of live and dead mites was determined after 24 h. A mite was considered dead if it did not move at least the length of its body when touched by a fine brush (No. 000). For each acaricide, the concentrations that promoted 0–100 % mortality of the *R. indica* specimens were determined. The same procedure was performed with *A. largoensis*; however, for the phytoseiid, only the five acaricides that proved to be toxic to *R. indica* were tested. During the bioassays, only castor bean pollen was used to feed the predators.

Bioassay

From the preliminary tests, 7–8 concentrations of each acaricide were established, ranging across the concentrations shown to result in 0–100 % mortality of the mites. The control treatment corresponded to the immersion of the leaf discs in distilled water. The applications of acaricides and the evaluations were performed in a manner similar to those described for the preliminary tests. The bioassays had three replications for each

concentration for a total of 30 adult females of *R. indica* and 15 adult females of *A. largoensis*, including the control. The entire procedure was repeated twice for a total 60 of and 30 mites per concentration for *R. indica* and *A. largoensis*, respectively.

Statistical analysis

The mortality data were submitted to a probit analysis (Finney 1971) after the correction of the mortality based on the control (Abbott 1925). The POLO-PC program (LeOra-Software 1987) was used to obtain the concentration response curves.

Results and discussion

The probit model fitted to mortality data of *R. indica* and *A. largoensis* (χ^2, $p > 0.05$). The estimated concentrations that caused 50–90 % mortality (LC_{50} and LC_{90}, respectively) indicated that abamectin, fenpyroximate, milbemectin, spirodiclofen and propargite were the products most toxic to *R. indica* (Table 1). Estimates of the toxicity ratios for the LC_{50} showed that milbemectin was 6,028,000 times more toxic than spiromesifen and 11 times more toxic than abamectin. Estimates of the toxicity ratios for the LC_{90} indicated that milbemectin was 263,000,000 times more toxic than chlorfenapyr and only 1.44 times more toxic than abamectin. The slopes of the concentration-mortality curves ranged from 0.39 to 1.69 for the acaricides chlorfenapyr and abamectin, respectively.

The LC_{50} of the acaricides tested for *A. largoensis* ranged from 0.092 to 895.98 mg/l for abamectin and propargite, respectively, and the LC_{90} ranged from 2.23 to 761,637 mg/l for milbemectin and propargite, respectively (Table 2). The relative selectivity (lethal concentration of the predator/lethal concentration of the pest) for the LC_{50} ranged from 1.553 to 47,338 for fenpyroximate and milbemectin, respectively, and the relative selectivity for the LC_{90} ranged from 1.933 to 59,739 for fenpyroximate and abamectin, respectively.

The efficacy these acaricides for the control of *R. indica* in the field cannot be determined based on the LC_{90} values estimated in this study. However, it is possible to compare the toxicity of the acaricides evaluated for the organisms in this study. Abamectin, hexythiazox, fenpyroximate and spirodiclofen are currently registered for use in Brazil to control *Aceria guerreronis* Keifer (Acari: Eriophyidae) on coconut palm, which is the main crop attacked by *R. indica* in the country. The field doses recommended by the pesticides manufacturers for the control of *A. guerreronis* are 13.5–100 mg/l for abamectin and fenpyroximate, respectively. These doses are much higher than the values of the LC_{90} estimated in this work for *R. indica*. The concentrations recommended of abamectin and fenpyroximate for the control of *A. guerreronis* may also be efficient to *R. indica* on coconut palms. In contrast, the LC_{90} estimated for *R. indica* were higher than the field doses recommended of hexythiazox (15 mg/l) and spirodiclofen (72 mg/l) for controlling *A. guerreronis*.

Field tests to evaluate the effectiveness of acaricides to *R. indica* have been performed in India and the Middle East (Sarkar and Somchoudhury 1988; Jalaluddin and Mohanasundaram 1990, Jayaraj et al. 1991), with the best results being obtained using dicofol, dimethoate, endosulphan, ethion, monocrotophos, phosphamidon, quinalphos and phosalone. However, the use of several of these acaricides is prohibited in Brazil (Ministério de Agricultura and Pecuária e Abastecimento 2012) and in many other countries due to their high toxicities. Recently, Rodrigues and Peña (2012) evaluated various acaricides under

Table 1 Toxicity of acaricides towards *Raoiella indica*

Active ingredient	n[a]	DF[b]	χ^2 [c]	Slope ± SE[d]	LC_{50} (95 %)[e]	TR^f_{50}	LC_{90} (95 %)[e]	TR^f_{90}
Milbemectin	420	5	1.09	0.77 ± 0.09	0.000004 (0.000001–0.000007)	–	0.00016 (0.0012–0.022)	–
Abamectin	480	6	1.63	1.69 ± 0.17	0.00004 (0.00003–0.00005)	11.20	0.00023 (0.00017–0.00033)	1.44
Spirodiclofen	540	7	3.17	0.55 ± 0.05	0.72 (0.27–1.59)	202,000	154.59 (67.09–447. 32)	966,000
Propargite	540	7	3.77	0.51 ± 0.05	0.88 (0.36–1.88)	246,000	297.12 (113.95–1087.37)	1,857,000
Fenpyroximate	479	6	4.89	0.94 ± 0.10	0.95 (0.50–1.58)	266,000	21.78 (12.83–44.00)	136,125
Diafenthiuron	540	7	8.14	0.58 ± 0.06	1.14 (0.31–2.94)	319,000	184.80 (69.95–721.11)	1,155,000
Fenbutatin oxide	420	5	3.59	0.56 ± 0.07	3.38 (1.25–7.29)	947,000	676.10 (249.95–3010.41)	4,225,000
Hexythiazox	540	7	3.58	0.44 ± 0.05	10.56 (2.77–29.21)	3,000,000	8403.79 (2597.79–44884.54)	52,000,000
Chlorfenapyr	540	7	6.43	0.39 ± 0.04	20.82 (8.001–48.450)	5,830,000	42,000 (10537.40–316411.62)	263,000,000
Spiromesifen	540	7	1.39	0.44 ± 0.05	21.52 (6.73–54.83)	6,028,000	18,000 (5371.09–107806.85)	114,000,000

[a] Total bioassay subjects

[b] Degrees of freedom

[c] Chi square value ($p > 0.05$)

[d] Standard error of the mean

[e] Concentrations in mg/l

[f] Toxicity ratio

Table 2 Selectivity of acaricides towards *Amblyseius largoensis*

Active ingredient	n[a]	DF[b]	χ^2 [c]	Slope ± SE[d]	LC_{50} (95 %)[e]	RS^f_{50}	LC_{90} (95 %)[e]	RS^f_{90}
Abamectin	240	7	1.19	0.59 ± 0.09	0.092 (0.027–0.225)	2,300	13.740 (4.160–110.540)	59,739
Fenpyroximate	225	6	4.65	0.88 ± 0.09	1.476 (0.845–2.632)	1.553	42.101 (18.897–129.794)	1.933
Milbemectin	210	5	1.52	1.14 ± 0.16	0.169 (0.089–0.284)	47,338	2.232 (1.213–89.746)	13,950
Propargite	240	6	3.53	0.79 ± 0.11	895.983 (478.274–1,765.155)	1,018	761,637 (13,047.51–204,909.772)	2,564
Spirodiclofen	240	6	5.23	0.90 ± 0.09	12.664 (7.552–21.590)	17.588	331.750 (154.990–974.919)	2.145

[a] Total bioassay subjects

[b] Degrees of freedom

[c] Chi square value ($p > 0.05$)

[d] Standard error of the mean

[e] Concentrations in mg/l

[f] Relative selectivity

field conditions in Puerto Rico and Florida (USA), and they found that acequinocyl, dicofol and spiromesifen were effective in reducing the populations of *R. indica*.

In our study, abamectin and milbemectin were the products most toxic to *R. indica*; however, abamectin showed the largest angular coefficient among the acaricides evaluated. Therefore, this acaricide is the most promising due its rapid and efficient control of the pest (it causes more than 90 % of mortality). The estimated LC_{90} of the products tested for *A. largoensis* indicated that abamectin and milbemectin were more toxic than fenpyroximate, propargite and spirodiclofen. Therefore, fenpyroximate, propargite and spirodiclofen are the most indicated for the management of *R. indica* because they are toxic to the pest and show a low toxicity to *A. largoensis*. Kim et al. (2005) evaluated the effect of abamectin on adult females of the predator *Neoseiulus cucumeris* (Oudemans) (Phytoseiidae) and concluded that this acaricide is very toxic to the phytoseiid. Noii et al. (2008) reported that the exposure to abamectin residues had a negative effect on *Phytoseius plumifer* (Canestrini & Fanzago). In the other hand, several studies consider abamectin a product that does not cause harm to phytoseiids. Zhang and Sanderson (1990) concluded that abamectin is considerably more toxic to *Tetranychus urticae* Koch compared with *Phytoseiulus persimilis* Athias-Henriot. Ibrahim and Yee (2000) reported that the reproductive performance, longevity and sex ratio of *Neoseiulus longispinosus* (Evans) was not affected by abamectin, and these authors recommended the use of this product for the management of *T. urticae*. Irigaray et al. (2007) evaluated the mortality of *Galendromus occidentalis* (Nesbitt) and *P. persimilis* caused by abamectin and found that this product had a small residual effect on these predators. These conflicting results may be explained by the differences in the susceptibility between populations to abamectin (Hamedi et al. 2011) or due to the methodologies used during the cited studies.

Phytophagous mites introduce stylets into the plant tissues during the feeding and through this structure they extract the contents of the leaf mesophyll cells (Moraes and Flechtmann 2008). *Raoiella indica* normally feeds on Arecaceae plants that have leaves with a thick, fibrous epidermis and are covered with wax. Ochoa et al. (2011) studied how *R. indica* and other species of the same genus feed on coconut palms, banana trees, *Heliconia* and eucalyptus and they found that all species of *Raoiella* fed by inserting stylets into the stomata between the guard cells of all of the plant species studied. The authors also claimed that *R. indica* avoids the mechanical defenses of the plant by exploiting a structural weakness in the architecture of the leaf. The results of the present study showed that the acaricides that were most toxic to *R. indica* were abamectin and milbemectin, which are activators of chloride channels. These acaricides have a translaminar action in the plant and act via the contact and ingestion by the arthropods (IRAC 2012). The other products tested also act via contact and ingestion; however, they do not have a translaminar action in the plant and have different modes of action, acting on respiration (chlorfenapyr, fenpyroximate, fenbutatin oxide, propargite and diafenthiuron) and the growth and development (spirodiclofen and spiromesifen) of the mites (Yu 2008). Therefore, it is possible that the insecticide–plant–mite interaction has a greater effect in controlling of *R. indica* by abamectin and milbemectin, due to the translaminar action of these products and the feeding behavior of *Raoiella* (stomatal feeding).

Spiromesifen and spirodiclofen act as inhibitors of lipid synthesis and also by contact and ingestion by the arthropods (IRAC 2012). These acaricides cause high mortalities in immature forms and affect female fertility but are not as effective for adult mortality (Nauen et al. 2005; Marčić et al. 2009, 2011). However, Rodrigues and Peña (2012) found that spiromesifen at a dose of 0.145 mg/l was effective in controlling both the adult and immature forms of *R. indica* on coconut palms. With the introduction of *R. indica* in Brazil

in 2009, the emergency registration of spiromesifen for the control of this mites on palm trees and Musaceae was conceded by the Ministry of Agriculture, Livestock and Supply for the period of September 2009 to September 2010. Chlorfenapyr, diafenthiuron and fenpyroximate have been reported to cause mortality in adult and immature mites and demonstrated intermediate toxicity among the products that were tested against *R. indica* in the present study. However, these compounds are not very toxic to *Neoseiulus womersleyi* (Schicha) (Kim and Seo 2001) and *P. persimilis* (Kim and Yoo 2002).

To date, no acaricide available on the Brazilian market is registered for control of *R. indica* in Brazil (Ministério de Agricultura and Pecuária e Abastecimento 2012), and the results obtained in this study may support the selection of acaricides to be used in the integrated pest management of *R. indica*. Further investigations should be conducted under field conditions to establish the appropriate time of year for the control and efficiency of these acaricides to manage properly the pest population and minimize the impacts on natural enemies.

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