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ORIGINAL ARTICLE

Effects of acaricides on *Oligonychus* sp. and compatibility with predatory mites *Neoseiulus californicus* and *Phytoseiulus persimilis*

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Abstract

In Mexico, outbreaks of phytophagous papaya mites (*Oligonychus* sp.) can threaten papaya production by damaging young leaves, causing a reduction in plant vigour and fruit yield. In the present study, we evaluated the effect of conventional acaricides on the papaya mite and on predatory mites *Neoseiulus californicus* and *Phytoseiulus persimilis*. In the laboratory, residual toxicity was tested by exposing *Oligonychus* sp. to acaricide-immersed leaves and the predatory mites to acaricide-coated glass vials. In the greenhouse and small field plots, mite-infested papaya leaves were sprayed with different acaricides to evaluate their control of *Oligonychus* sp. In the laboratory, abamectin, spirodiclofen, and fenpyroximate caused the highest mortality (95–100%) of *Oligonychus* sp. adults and nymphs on day 1 after application. These acaricides also caused 100% adult mortality of both predatory mites. Spirodiclofen and etoxazole killed all eggs of *Oligonychus* sp. In the greenhouse and small field plots, abamectin, spirodiclofen, and fenpyroximate were effective at controlling the population of adults, nymphs, and eggs of *Oligonychus* sp.

Keywords Phytophagous mites · Acaricide effectiveness · Pest management

Introduction

Phytophagous mites are among the most destructive pests of papaya (*Carica papaya* L.). Mites can significantly reduce photosynthesis and transpiration, affecting plant growth and fruit yield (Helle and Sabelis 1985; Huffaker et al. 1969). Two of the most damaging species of papaya are the two-spotted spider mite (*Tetranychus urticae* Koch) and the broad mite (*Poliphagotarsonemus latus* Banks) (Valencia-Dominguez et al. 2011; Abato-Zárate et al. 2014). Whereas *T. urticae* feeds on mature leaves, causing yellowing, *P. latus* affects young leaves, causing chlorosis, deformation, and smaller blades (Abato-Zárate et al. 2018; Acuña et al. 2005; Alcántara et al. 2011). Other mite species cause large losses in specific papaya-growing regions, for example, *Tetranychus kanzawai* Kishida and *Tetranychus cinnabarinus* Boisduval in Taiwan (Lu and Wang 2005), *Tetranychus merganser* Boudreaux in southern Mexico (Valencia-Domínguez et al. 2011), and *Oligonychus indicus* Hirst in West India (Goshal et al. 2010). In recent years, papaya growers in southern Mexico have faced high infestations of *Oligonychus* sp. that caused yellowing, malformation, and necrosis of young leaves, which resulted in a dramatic decrease in fruit yield (Cua-Basulto 2018).

Tetranychus urticae, *T. merganser,* and *P. latus* are effectively controlled on papaya by the acaricides dicofol, bifenthrin, spiromesifen, abamectin, fenazaquin, paraffinic oil, sulphur and fatty acid salts (Acuña et al., 2005; Abato-Zárate et al., 2012; Herrera-Palacios et al., 2018), but no studies have evaluated acaricides for controlling *Oligonychus* sp. on papaya. In addition to the use of acaricides, the inclusion of biological control agents also holds promise for controlling mites. For example, the Phytoseiidae *Phytoseiulus persimilis* Athias-Henriot and *Neoseiulus californicus* McGregor have been successful as biological control agents against *T. urticae* and *T. merganser* on papaya plantations in Colombia and Mexico, which highlights the importance of these predatory

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mite species (López-Bautista et al. 2016; Mena et al. 2020). Even though these species have not been observed as natural acarofauna on papaya, other species of Phytoseiidae has been reported in various regions; *Typhlodromalus* spp., *Amblyseius* spp., and *Neoseiulus idaeus* Denmak & Muma are present in Brazil's southeastern tropical coastal plain (Collier et al. 2004), *Neoseiulus fallacis* Garman in Indonesia (Puspitarini et al. 2019), and *Amblyseius* spp. and *Neoseiulus longispinosus* Evans in Cuba (Díaz-Tejeda et al. 2010).

Here, we evaluated the effects of conventional acaricides on the papaya mite *Oligonychus* sp. in the laboratory, greenhouse and in small field plots and on two predatory mites *N. californicus* and *P. persimilis* in the laboratory.

Material and methods

Mite sources

Oligonychus sp. was collected from papaya fields in Tepakan, Yucatán. The mite colony was established on 3–5-month-old papaya plants in a greenhouse at 25–32 °C and 70–90% RH with a photoperiod of 14 h light:10 h dark. The predatory mites *P. persimilis* and *N. californicus* were purchased from Koppert Biological Systems (Queretaro, Mexico).

Molecular identification of Oligonychus sp

For the molecular identification of *Oligonychus* sp., mitochondrial DNA (mtDNA) was extracted separately from three samples with hexadecyltrimethyl ammonium bromide (2% with 5 M NaCl), according to the protocol of Doyle and Doyle (1987) as modified by the Ministry of Agriculture, Livestock, Rural Development, Fisheries and Food (SAGARPA-Mexico, 2017). The quantity and quality of DNA were estimated using a NanoDrop 2000® spectrophotometer (Thermo Scientific, Waltham, MA, the USA). DNA integrity was analyzed by electrophoresis in 0.8% (w/v) agarose gels in 1X Tris–borate–EDTA buffer (TBE) and ethidium bromide staining. PCR amplifications were performed using universal primers, based on genomic regions of the cytochrome oxidase *c* subunit 1 (*COI*) gene, to amplify a 710-bp DNA fragment (LCO1490 5' GGTCAACAAATC ATAAAGATATTGG 3'; HC02198 5' TAAACTTCAGGG TGACCAAAAAATCA 3') (Folmer et al. 1994). The following cycling conditions were used: 1 min denaturation at 94 °C; 4 cycles of 94 °C for 30 s, 45 °C for 90 s, and 72 °C for 1 min; and 35 cycles of 94 °C for 30 s, 51 °C for 90 s, and 72 °C for 10 min. PCR products were visualized by electrophoresis in 1.5% (w/v) agarose gels in 1X TBE and ethidium bromide staining. PCR products were purified using the Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, the USA) and were sequenced in both directions by Macrogen Inc., South Korea. Sequences obtained were analyzed using the Basic Local Alignment Search Tool (BLAST), accessed via the National Centre for Biotechnology Information (NCBI) website.

Tested acaricides

Five acaricides recommended for the control of *T. urticae* in papaya were evaluated in the laboratory and greenhouse at concentrations recommended by the manufacturer (Table 1). The three most effective acaricides were also tested in small field plots. The control was only sprayed with distilled water.

Laboratory bioassays

Acaricides were tested against adults, nymphs, and eggs of *Oligonychus* sp. In bioassays of adults and nymphs as described by Monteiro et al. (2015), papaya leaf discs (5 cm in diameter) were cut and immersed for 5 s in a 100-mL beaker containing different acaricide solutions. Treated leaf discs were dried for 30 min at room temperature and then placed adaxial side up on moistened cotton in Petri dishes (9 cm diameter, 1.5 cm depth). The edges of the leaf disc were covered with moistened cotton to prevent mites from escaping. Fifteen mites were transferred onto each leaf disc, and mortality was recorded after 1, 2, and 3 days (Uddin et al. 2015). Mites that did not move after being touched with a fine brush were considered dead. Dead mites were removed from the leaf disc. A Petri dish represented a replicate. Eight replicates were included for each acaricide.

Egg mortality was evaluated as described by Wang et al. (2016). Adult females were transferred to papaya leaf discs

Table 1 Names and recommended doses of acaricides evaluated in the present study

Acaricide	Recommended dose (mg a.i./L)	Trade name	Manufacturer
Bifenazate	1000	Acramite 50% WS	Arysta Lifescience, Mexico
Etoxazole	200	Tetrasan 10.34% SC	Valent de Mexico S.A. de C.V
Fenpyroximate	100	Sumatrus 5% SC	Agroquimicos Versa S.A. de C.V
Spirodiclofen	500	Envidor 22.30% SC	Bayer Crop Science Mexico S.A. de C.V
Abamectin	36	Abakrone 1.80% EC	BioKrone Mexico, S.-A. de C.V

(30 females per 5-cm-diameter disc) placed on moistened cotton in Petri dishes (9 cm in diameter). After 24 h, all adults were removed. Some eggs were also removed to leave only 20 eggs per leaf disc. Leaf discs containing the eggs were carefully taken with forceps and dipped 5 s into the acaricide solutions. Subsequently, treated leaf discs were dried for 30 min at room temperature and placed back into the Petri dishes. Petri dishes were maintained at room temperature (24–30 °C) for 6 days. Egg mortality was then recorded; eggs that did not hatch were considered dead. A Petri dish represented a replicate. Five replicates were included for each acaricide treatment.

The toxicity of the acaricides on the predatory mites (*N. californicus* or *P. persimilis*) was evaluated as described by Cheri et al. (2013). Acaricide solutions (5 mL) were poured into 20-mL glass vials and swirled for even coverage over the glass surface; the excess was discarded, and the vials placed in a fume hood for 3 h. Then, a drop of honey was streaked inside each vial. Subsequently, ten adults of *N. californicus* or *P. persimilis* were placed into a vial and capped. Mortality of mites, taken as the lack of movement when disturbed, was recorded after 2 d. For this test, a vial represented a replicate. Five replicates were included for each acaricide.

Greenhouse and small field tests

The acaricides were tested against *Oligonychus* sp. in a greenhouse and field in Conkal, Yucatan, Mexico. In a greenhouse with a plastic ceiling and anti-aphid lateral mesh, 3-month-old papaya plants were infested with *Oligonychus* sp. by placing a heavily infested papaya leaf on each shoot. After 21 days, when the mite colony was established, the acaricides were sprayed with a hand sprayer until runoff. A plant represented a replicate, with 10 replicates per acaricide treatment. Mites in each population were counted before spraying (day 0) and at 1, 7, and 14 days after acaricide application by excising one fully expanded young leaf on the upper third of each plant, taking it to the laboratory and counting the number of adults, nymphs, and eggs using a stereoscope (40x). The leaf area was measured with a LI-COR area meter model LI-3100C (Lincoln, NE, the USA).

For the small field evaluation, the plots were arranged in a randomized complete block design with four replicates. Each experimental plot contained 15 plants spaced at 2.5×2 m. Eight months after transplanting, plants were infested with *Oligonychus* sp. as described for the greenhouse evaluation. After 30 days, when the mite colonies were established on the young leaves, whole plants were sprayed with abamectin, spirodiclofen, or fenpyroximate, using a 25-L backpack sprayer. Mite numbers and leaf areas were recorded before spraying (day 0) and at 1, 7, 14, 21, and 28 days after acaricide application as described for the greenhouse evaluation. In each experimental plot, three plants in the central area were selected, and one fully expanded young leaf of the upper third of each plant was sampled for a total of 12 leaves (replicates) per treatment.

Data analysis

Mortality data for *Oligonychus* sp. nymphs and adults at days 1, 2 and 3 after acaricide application in the laboratory were subjected to analysis of variance with repeated measures and Bonferroni post hoc test. Mortality data for *Oligonychus* sp. eggs were subjected to one-way analysis of variance and Tukey post hoc test.

Mortality data for the predatory mites (*N. californicus* and *P. persimilis*) were analyzed by a factorial analysis of variance to detect any effect of the acaricides within a single mite species and compare the effects of the acaricides between species.

Data for the population density of *Oligonychus* sp. on papaya were subjected to randomized block analyses of variance and Tukey post hoc test.

In all cases, data were first checked for normality and homoscedasticity. The effects were considered statistically significant if $P < 0.05$. All analyses were performed in the Statistical Package for Social Scientists version 16.0 (SPSS, Chicago, IL, the USA).

Results

Molecular identification

The PCR using LCO1490 and HC02198 primers yielded a single band of 710 bp from the collected samples. The BLAST search of the genetic sequence in the GenBank database showed 90–92% of similarity with *Oligonychus* sp. (Fig. 1).

Toxicity of acaricides to Oligonychus sp. and predatory mites in the laboratory

All acaricides caused significant mortality of *Oligonychus* sp. adults ($F = 813.8$; df = 5, 42; $P < 0.001$) and nymphs ($F = 1560.7$; df = 5, 42; $P < 0.001$). For adults, abamectin caused 100% mortality on day 1 after application (Fig. 2). Spirodiclofen caused more that 90% mortality at day 1, and at day 2, the mortality was 100%. Fenpyroximate caused less than 50% mortality at day 1, but reached 100% at day 2. Bifenazate and etoxazole caused low mortality at day 1 (< 20%), but mortality increased significantly at day 2 and day 3 after application (Fig. 2). For nymphs, abamectin, spirodiclofen and fenpyroximate caused 100% mortality on day 1 after application. Bifenazate and etoxazole also

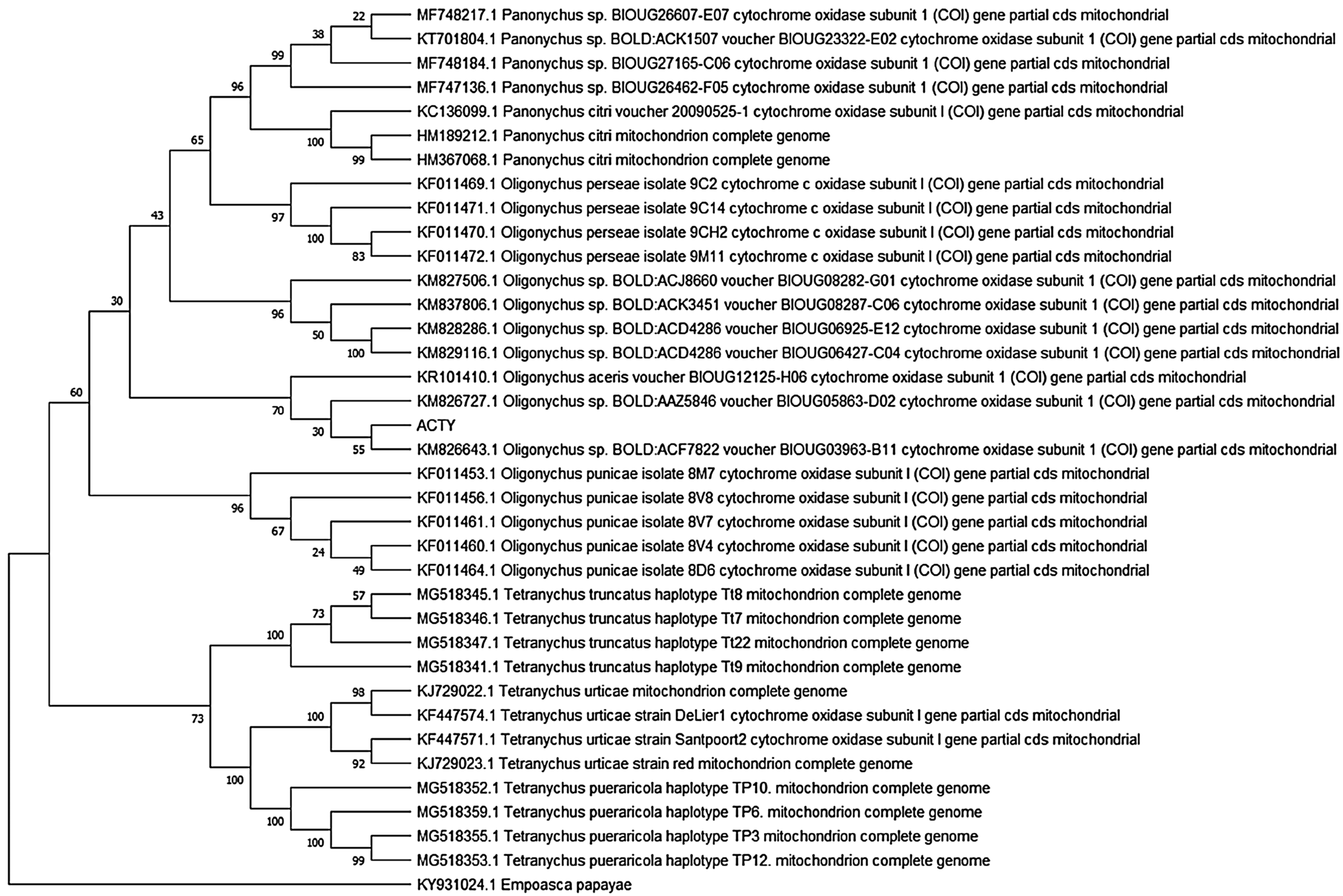

Fig. 1 Phylogenetic tree derived from cytochrome oxidase c subunit 1 (*COI*) gene using the neighbour-joining method and showing the position of *Oligonychus* sp. (ACTY). GenBank accessions are given at the start of each entry. Bootstrap values were determined from 1000 iterations and are shown on the branches

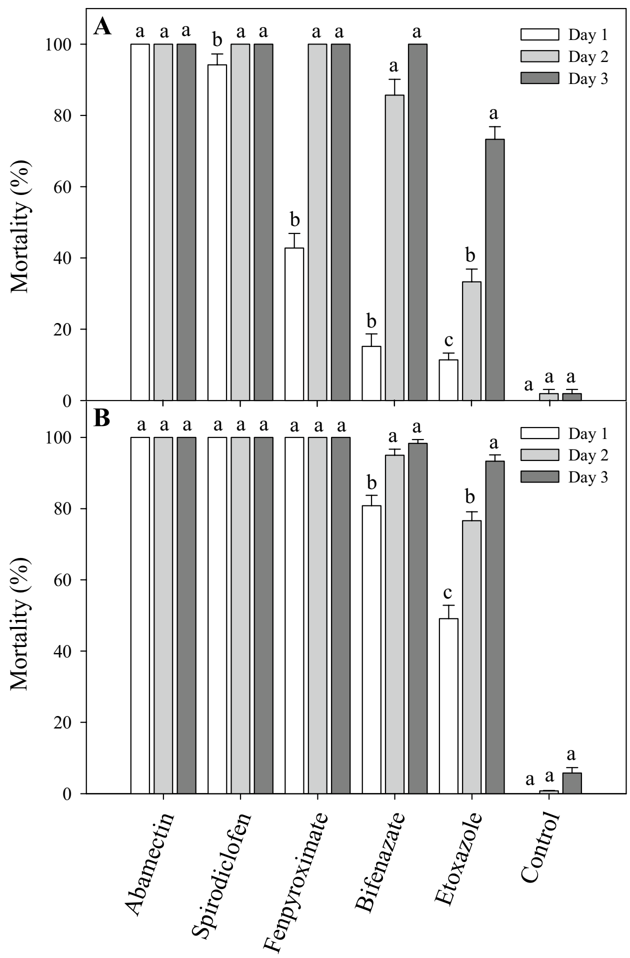

Fig. 2 Mortality of papaya mite *Oligonychus* sp. adults **(a)** and nymphs **(b)** exposed for 3 days to the acaricides in the laboratory. Means for the same chemical that do not share the same letter differed significantly (repeated measures ANOVA, Bonferroni post hoc test, $P<0.05$)

caused high mortality (> 90%) but not until day 3 after application (Fig. 2).

The mortality of *Oligonychus* sp. eggs was significantly different among treatments ($F=797.9$; df = 5, 42; $P<0.001$). The highest mortality was produced by spirodiclofen and etoxazole (100%), followed by fenpyroximate (93%), bifenazate (84%), and abamectin (77%) (Fig. 3).

The acaricides also caused significant mortality to the predatory mites. Factorial analysis showed that the acaricides had differential effects ($F=1489.39$; df = 5, 59; $P<0.0001$). The highest mortality was produced by abamectin, spirodiclofen, and fenpyroximate (100%), followed by bifenazate (88–92%) and etoxazole (36–43%) (Fig. 4). The effect of the acaricides was similar for both predatory mite species; no difference was observed in their susceptibility ($F=413$; df = 1, 59; $P>0.05$).

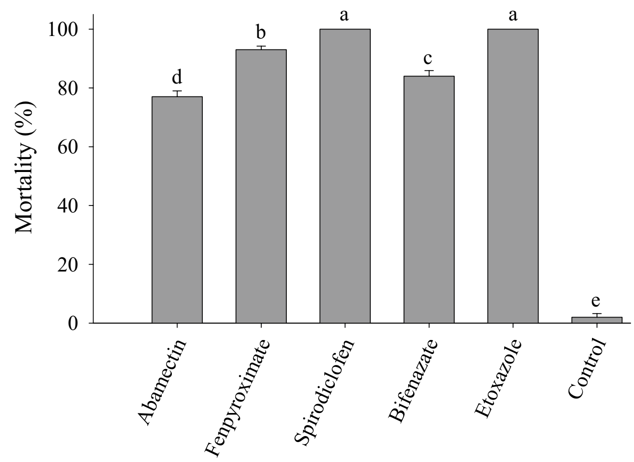

Fig. 3 Mortality (mean ± standard error) of *Oligonychus* sp. eggs 6 days after treatment with acaricides in the laboratory. Means that do not share the same letter differed significantly (one-way ANOVA, Tukey post hoc test, $P<0.05$)

Effect of the acaricides on mite population in the greenhouse

All acaricides significantly reduced the population density of *Oligonychus* sp. on papaya leaves (Fig. 5). For adults, abamectin, spirodiclofen, and fenpyroximate showed the highest effectiveness at day 1 after spraying (Fig. 5a; $F=150.9$; df = 5, 54; $P<0.001$). These acaricides, in addition to bifenazate, were also the most effective at day 7

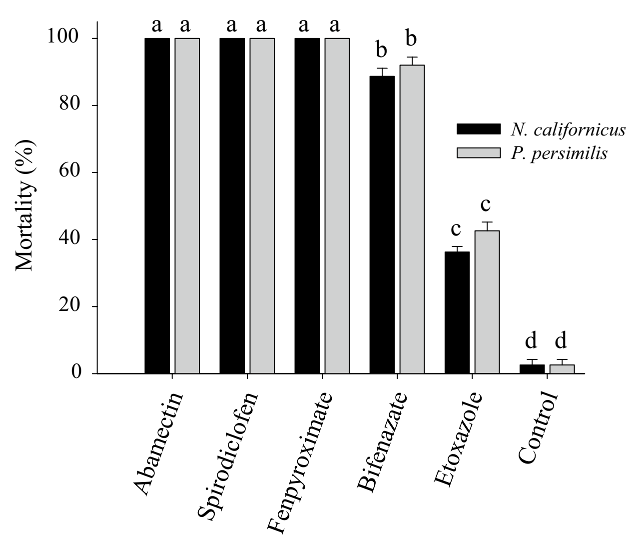

Fig. 4 Mortality (mean ± standard error) of *Neoseiulus californicus* and *Phytoseiulus persimilis* adults 2 days after exposure to the acaricides under laboratory conditions. Means that do not share the same letter differed significantly (factorial ANOVA, Tukey Post hoc test, $P<0.05$)

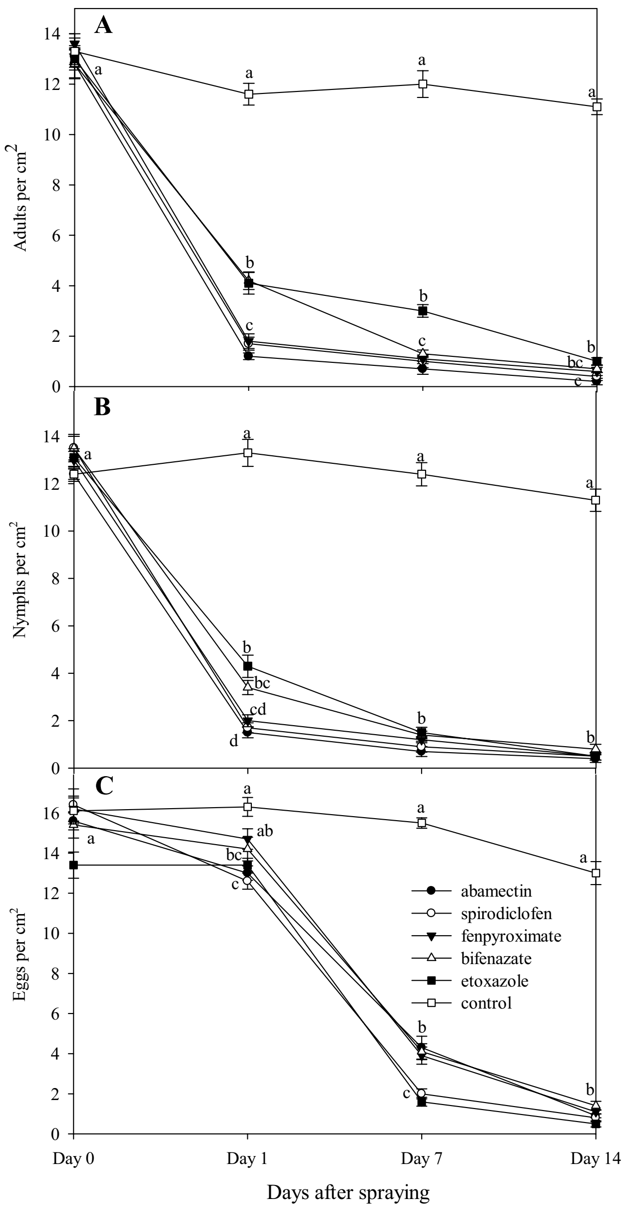

Fig. 5 Population density (mean ± standard error) of adults **(a)**, nymphs **(b)** and eggs **(c)** of papaya mite *Oligonychus* sp. on test leaf after application of the acaricides in a greenhouse. Means for the same day after spraying that do not share the same letter differed significantly (one-way ANOVA, Tukey post hoc test, $P<0.05$)

($F=231.3$; df = 5, 54; $P<0.001$) and day 14 ($F=515.5$; df = 5, 54; $P<0.001$).

For nymphs, abamectin, spirodiclofen, and fenpyroximate showed the highest effectiveness at day 1 after spraying (Fig. 5b; $F=150.0$; df = 5, 54; $P<0.001$), day 7 ($F=287.2$; df = 5, 54; $P<0.001$), and day 14 ($F=310.4$; df = 5, 54; $P<0.001$). For eggs, the acaricides showed low effectiveness at day 1 after spraying (Fig. 5c; $F=8.8$; df = 5, 54; $P<0.001$), but at day 7 ($F=182.3$; df = 5, 54; $P<0.001$), spirodiclofen and etoxazole had significantly reduced the population density of papaya mite eggs (1.6 to 2.0 eggs cm^2). At day 14 ($F=310.6$; df = 5, 54; $P<0.001$), all acaricides showed the same effectiveness in reducing the egg population of papaya mite (0.5 to 1.4 eggs cm^2).

Effects of the acaricides on the mite population in the small field experiments

In the small field tests of abamectin, spirodiclofen, and fenpyroximate, all acaricides significantly reduced the population of adults, nymphs, and eggs of *Oligonychus* sp. (Fig. 6). For adults, abamectin and spirodiclofen showed the highest effectiveness at all dates (Fig. 6a; 1, 7, 14, 21, and 28 days after spraying). Fenpyroximate was also effective against the adult population, but its effect was lower than that of abamectin and spirodiclofen.

For nymphs, abamectin and spirodiclofen had the highest effectiveness at day 7 after spraying (Fig. 6b). At days 21 and 28 after spraying, all acaricides had similar efficacy against the nymph population.

For eggs, all the acaricides showed slight but significant effects at day 1 (Fig. 6c). However, by day 7, abamectin and spirodiclofen had significantly reduced the population density. At day 14, all acaricides had maintained their effectiveness to the same extent. At day 21, the population density of eggs had increased, but the effect of the acaricides was still significant relative to the control. At day 28, the acaricides had no effect on the population density of eggs.

Discussion

In this evaluation of the effects of acaricides on the papaya mite *Oligonychus* sp. and the two predatory mites *N. californicus* and *P. persimilis*, the toxicity tests in the laboratory showed that abamectin, spirodiclofen, and fenpyroximate were highly toxic to adults and nymphs of *Oligonychus* sp. and to adults of *N. californicus* and *P. persimilis*. Etoxazole and spirodiclofen were also highly toxic to eggs. In the greenhouse and small field plots, abamectin, spirodiclofen, and fenpyroximate effectively controlled the population of *Oligonychus* sp.

Overall, abamectin, spirodiclofen, and fenpyroximate were the most effective acaricides to control *Oligonychus* sp. These acaricides have different modes of action: abamectin targets the gamma-aminobutyric receptors on chloride channels, spirodiclofen targets acetyl-CoA-carboxylase, and fenpyroximate inhibits mitochondrial NADH-Co Q reductase (Ishaaya et al. 2007; Marcic 2012; Reddy et al. 2014). Having an arsenal of acaricides with different modes of action to control the papaya mite *Oligonychus* sp. is important for developing a comprehensive management programme to prevent or delay resistance to acaricides.

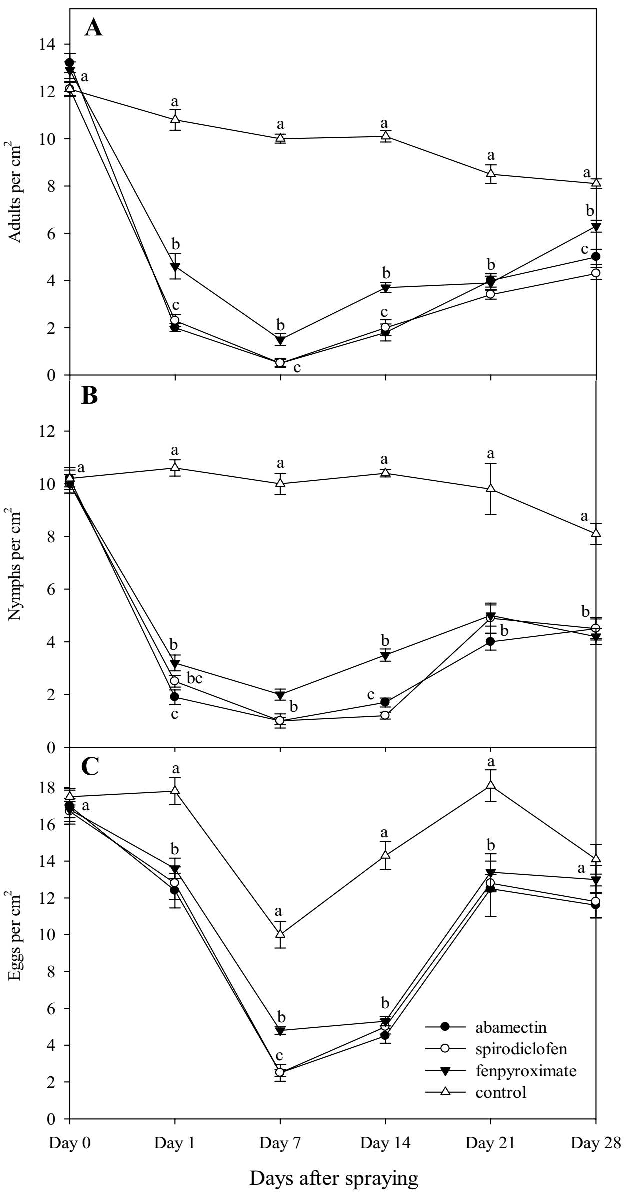

Fig. 6 Population density (mean ± standard error) of adults **(a)**, nymphs **(b)** and eggs **(c)** of papaya mite *Oligonychus* sp. on a test leaf after application of the acaricides in a small field plots. Means for the same day after spraying that do not share the same letter differed significantly (one-way ANOVA, Tukey post hoc test, $P < 0.05$)

Abamectin, one of the most-used acaricides in agriculture, has shown high effectiveness against different tetranychid species, including *Oligonychus* sp. (Roy et al. 2012, Ramos-Gutiérrez et al. 2015). It has been extensively used because it has a strong toxic effect within a few hours (4 h) after application (Uddin et al. 2015) and a fair residual effect in the field (up to 3 weeks) when applied at recommended field rates (Duchovskiene 2009, Uddin et al. 2015).

Spirodiclofen has been used effectively against a wide range of phytophagous mites (Raudonis 2006; Ouyang et al. 2011; Lemus-Soriano and Pérez-Aguilar 2016). Although its effect is not as rapid as that of a neurotoxic acaricide (Raudonis 2006), we observed strong effects (100% mortality) against *Oligonychus* sp. by day 1 after application.

Fenpyroximate has been effective in controlling species of the Tetranychidae family (Reddy et al. 2014; Kumari et al. 2015), including *Oligonychus* spp. (Aswin et al. 2015; Lemus-Soriano and Pérez-Aguilar 2016). Contrary to previous reports that fenpyroximate exerts its effect within 2 to 3 days after application (Kumari et al. 2015; Wang et al. 2018), we found that it produced 100% mortality against nymphs by day 1 after application.

When selecting an acaricide for a pest management programme, one of the main aspects to consider is the persistence of the effects. In this sense, abamectin and spirodiclofen are considered acaricides with long persistence (3 to 4 weeks) when applied at field recommended rates (Duchovskiene 2009, Huerta-Pérez et al. 2017). The persistence of fenpyroximate is substantially lower (from 1 to 3 weeks; Lemus-Soriano and Pérez-Aguilar 2016; Wang et al. 2018).

In the present study, spirodiclofen and etoxazole showed strong ovicidal effects (100% mortality), agreeing with previous reports that these acaricides have ovicidal effects within 4 weeks after spraying in the field (Dekeyser 2005; Ochiai et al. 2007; Montoya et al. 2017). It is important to point out that in the field, a decrease in egg population in the long term may not only be due to a direct ovicidal effect, but also to fewer eggs being laid by the reduced population of adults.

We also found that abamectin, spirodiclofen, and fenpyroximate were highly toxic to the predatory mites *N. californicus* and *P. persimilis*, in agreement with studies that documented the lethal effects of abamectin and spirodiclofen on *P. persimilis* (Bostanian and Akalach 2006; Duso et al. 2008) and *N. californicus* (Kaplan et al. 2012; Assis et al. 2018). Although fenpyroximate has not been tested against *N. californicus* or *P. persimilis*, highly toxic effects have been documented against other predatory mite species (Irigaray and Zalom 2006; Nadimi et al. 2011). Knowing the potential risk of acaricides against predatory mites should provide a basis for selecting chemical controls that are compatible with biological control agents in integrated pest management programmes.

In summary, to control *Oligonychus* sp. in papaya, abamectin, spirodiclofen, and fenpyroximate were highly effective against the motile stages. In addition, spirodiclofen and etoxazole had strong ovicidal effects. However, these acaricides were also highly toxic to the predatory mites *P. persimilis* and *N. californicus* following residual exposure in the laboratory. Even though these data from the laboratory do not necessarily reflect a realistic situation in the field, our results suggest that the use of abamectin, spirodiclofen, and fenpyroximate might have a significant negative impact on the predatory mites *P. persimilis* and *N. californicus* and probably other predatory mite species in papaya fields.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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