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Population-level effects of abamectin, azadirachtin and fenpyroximate on the predatory mite *Neoseiulus baraki*

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Abstract The coconut production system, in which the coconut mite *Aceria guerreronis* is considered a key pest, provides an interesting model for integration of biological and chemical control. In Brazil, the most promising biological control agent for the coconut mite is the phytoseiid predator *Neoseiulus baraki*. However, acaricides are widely used to control the coconut mite, although they frequently produce unsatisfactory results. In this study, we evaluated the simultaneous direct effect of dry residue contact and contaminated prey ingestion of the main acaricides used on coconut palms (i.e., abamectin, azadirachtin and fenpyroximate) on life-history traits of *N. baraki* and their offspring. These acaricides are registered, recommended and widely used against *A. guerreronis* in Brazil, and they were tested at their label rates. The offspring of the exposed predators was also evaluated by estimating the instantaneous rate of population increase (r_i). Abamectin compromised female performance, whereas fenpyroximate did not affect the exposed females (F0). Nonetheless, fenpyroximate strongly compromised the offspring (F1) net reproductive rate (R_0), intrinsic rate of population growth (r_i), and doubling time (DT). In contrast, fenpyroximate did not have such effects on the 2nd generation (F2) of predators with acaricide-exposed grandparents. Azadirachtin did not affect the predators, suggesting that this acaricide can be used in association with biological control by this predatory species. In contrast, the use of abamectin and fenpyroximate is likely to lead to adverse consequences in the biological control of *A. guerreronis* using *N. baraki*.

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Introduction

Predatory mites are considered effective natural enemies of phytophagous mites (Helle and Sabelis 1985; McMurtry and Croft 1997; McMurtry et al. 2013). Many predatory mite species are specialized feeders and are able to respond to the population dynamics of particular species (Solomon et al. 2010), such as specialized mite predators (type I predators according to McMurtry et al. 2013). However, they are frequently unable to fully and reliably prevent pest damage when used as a sole management tactic (Solomon et al. 2010). In such cases, alternative strategies are needed to control mite pests. The control of phytophagous mites is frequently performed almost exclusively using acaricides (Watanabe et al. 1994). Nevertheless, this is an expensive management tactic that requires periodic acaricide applications and the purchase of suitable equipment and supplies. Thus, given its high cost, the use of acaricides can be economically prohibitive for small-scale farmers. In such low-input production systems (systems that receive no or low cash inflows), the integration between predatory mites and acaricides becomes an economically viable option. For this integration to be possible, acaricides with low (or no) negative impacts on predatory mites are required. As the coconut production system permits the potential integration of these management tactics, it is a suitable model for studying such integration.

The coconut *Cocos nucifera* L. suffers from attack by several pests. Among these, the coconut mite *Aceria guerreronis* Keifer (Acari: Eriophyidae) is considered a key pest of coconut palms (Moore and Howard 1996; Haq et al. 2002). Several countries have reported losses of up to 60 % due to this pest (Negloh et al. 2011; Navia et al. 2013; Rezende et al. 2016). The coconut mite causes damage by feeding on the fruit perianth, an enclosed region under the bracts where the mite develops. The fruit surface becomes necrotic and can eventually result in premature fruit fall, reducing the number of fruits in the bunch (Moore and Howard 1996). The most common method of control for this pest is based on intensive acaricide use, but because this pest lives well protected under the floral bracts, the efficacy achieved using acaricides is not always satisfactory (Moore and Howard 1996; Ramaraju et al. 2002; Monteiro et al. 2012; Silva et al. 2016). Monteiro et al. (2012) suggest that control of the coconut mite using pesticides should only be implemented while the mites are dispersing by walking over sprayed fruit.

Biological control is gaining attention as a management alternative for coconut mites (Domingos et al. 2010; Lima et al. 2012). The most promising biological control agent for the coconut mite is *Neoseiulus baraki* (Athias-Henriot) (Acari: Phytoseiidae) (Aratchige et al. 2007; Negloh et al. 2008; Domingos et al. 2010; Melo et al. 2011; Lima et al. 2012). This predator has easier access to the microhabitat inhabited by the pest compared with other predators, and it exhibits a progressively higher predation rate of the coconut mite (Lima et al. 2012). *Neoseiulus baraki* has been tested in field inundative releases by Fernando et al. (2010), who reported that a single release of *N. baraki* in coconut palms provides significant reduction of the coconut mite population.

Within this context, understanding the potential effects of the acaricides used against the coconut mite on its predator *N. baraki* is fundamental for the integration of chemical and

biological control. Fenpyroximate and chlorfenapyr appear to be selective to *N. baraki* over *A. guerreronis* (Lima et al. 2013a). However, there is no information on the potential impacts of acaricides on the life history traits and demography of *N. baraki*. Information on demographic toxicology involves the ecological and toxicological parameters that predict the overall effect of toxins at the population level (Ahmadi 1983; Stark and Wennergren 1995; Guedes et al. 2016). Fecundity and population growth have been used as indicators of mite population performance. The latter is a more robust toxicological endpoint than typical mortality assessments because it includes assessments of fertility and the mortality and birth rates in a given population (Saito 1979; Sabelis 1985; Stark and Wennergren 1995; Stark et al. 1997; Ansaloni et al. 2007; Walthall and Stark 1997). The evaluation of the life table parameters of predators after exposure to acaricides is helpful for the selection of suitable acaricides with minimal non-target toxicity. In this study, we evaluated the effects of widely used acaricides (abamectin, azadirachtin and fenpyroximate) that are recommended by the Brazilian Ministry of Agriculture against *A. guerreronis* (Agrofit 2015) on the life history traits of *N. baraki* and their 1st (F1) and 2nd (F2) generation offspring.

Materials and methods

Establishment and maintenance of predator colonies

Coconuts were collected from the coastal island of Itamaracá ("Ilha de Itamaracá"), State of Pernambuco, Brazil (07°46′S, 34°52′W), and transported to the laboratory. The area is not commercially used; thus, it has not been subjected to pesticide applications or coconut mite control for over 10 years. Coconuts and mites were collected from 10 plants in the area. The fruits were maintained under controlled laboratory conditions (27 ± 1 °C, 70 ± 10 % relative humidity [RH] and a 12 h photoperiod). *Neoseiulus baraki* colonies were established using approximately 100 females collected from coconut fruits and transferred to rearing units. Each rearing unit consisted of a black PVC disk (13 cm in diameter, 1 mm thick) laid on a foam mat disk that lined the bottom of a plastic tray. The margin of the PVC disk was covered using a band of hydrophilic cotton, and both the foam mat and the cotton band were kept wet by daily additions of distilled water to the tray. *Aceria guerreronis* was provided as food on a piece of perianth (approximately 1 cm^2) obtained from the collected coconut fruits. Three hundred coconut mites were placed on each piece of perianth, which was replaced every 2 days to prevent the perianth from drying out, which would cause the coconut mites to starve to death. Five pieces of perianth were placed in each rearing unit. The units were maintained in a rearing chamber under the environmental conditions described above.

Acaricides

Azadirachtin, fenpyroximate and abamectin are registered and recommended by the Brazilian Ministry of Agriculture for use against *A. guerreronis* and widely used by coconut farmers (Agrofit 2015). These compounds were used in their respective commercial formulations as follows: azadirachtin (Azamax, 1.2 g a.i. [active ingredient]/L, emulsifiable concentrate, DAV Agro, Ituverava, SP, Brazil), fenpyroximate (Ortus, 50 g a.i./L, suspension concentrate, Arysta Lifescience, Salto de Pirapora, SP, Brazil), and

abamectin (Vertimec, 18 g a.i./L, emulsifiable concentrate, Syngenta, São Paulo, SP, Brazil). The acaricides were tested at a single rate, the maximum label rate for the coconut mite in Brazil (Agrofit 2015), and their corresponding concentrations used in our experiments were 30 mg a.i./L for azadirachtin, 100 mg a.i./L for fenpyroximate, and 13.5 mg a.i./L for abamectin (Agrofit 2015).

Effects of acaricides on the life history traits of the treated unmated females (F0)

Pieces of coconut perianth (0.5 cm^3), individual wells (i.e., cells) of bioassay trays and adhesive covers (128 cells; Bio-Serv, Frenchtown, NJ, USA) were immersed in water (control) or one of each of the acaricide solutions recommended for the coconut mite for 5 s and allowed to air-dry for 2 h (Lima et al. 2013a). Then, a treated piece of perianth was placed in each cell and 300 *A. guerreronis* were transferred onto each treated piece of perianth food source for the predators. One *N. baraki* female (12 h old) was transferred to each well and confined for 24 h. Treated adhesive covers were used to seal each well to prevent the mites from escaping. The wells were placed in a rearing chamber and maintained at 27 ± 1 °C and 70 ± 10 % RH and a 12 h daily photoperiod. After 24 h, the surviving females were transferred to untreated wells. An untreated male was also transferred to each (untreated) well containing a female to allow mating. The food was replenished every day with a new piece of perianth with coconut mites. Forty replicates were performed for each acaricide following a completely randomized design where each female represented one replicate.

Fertility and the pre-oviposition, oviposition, and post-oviposition periods were monitored, and data were recorded daily until female death. The males that died were replaced with new ones. For each treatment, the number of eggs per female (m_x) on each oviposition date (x) was calculated considering the total number of females, the cumulative survival rate of females (l_x) during the oviposition period, and the number of adult offspring of x age in the next generation ($l_x.m_x$). Using this information (m_x, l_x and $l_x.m_x$), the following parameters were estimated: net reproductive rate ($R_0 = \sum l_x m_x$), mean generation time ($T = \frac{\sum x l_x m_x}{\sum l_x m_x}$), intrinsic rate of increase ($r_m = \frac{Ln(R_0)}{T}$) and doubling time ($DT = \frac{Ln(R_0)}{r_m}$); the latter refers to the time needed to double the initial population.

The fertility and the pre-oviposition, oviposition, and post-oviposition periods were analysed using the Kruskal–Wallis test with the NPAR1WAY procedure (SAS Institute 2008). The survival data were used to construct time-mortality curves using Kaplan–Meier estimators with the LIFETEST procedure (SAS Institute 2008), and log-rank tests were used for pairwise planned comparisons. The median survival times were analysed using the Kruskal–Wallis test employing the NPAR1WAY procedure (SAS Institute 2008). The "jackknife" technique was used to estimate the confidence intervals to compare life table parameters (Maia et al. 2000). A χ^2 analysis was performed to determine whether there was any deviation from the expected sex ratio of 1:1 using the FREQ procedure (SAS Institute 2008).

Effects of acaricides on the development and reproductive performance of the offspring (F1) of treated females

Eggs from untreated (control) and treated females were transferred individually into untreated wells. Each subsequent stage was checked daily, and the developmental time and survival were recorded. When the adults emerged, they were sexed, and the females were

separated. Males were subsequently added and paired with the females. Coconut mites were provided as food on a piece of perianth (approximately 1 cm^2) containing nearly 300 individuals. The food was changed every day.

The developmental time (from egg to adulthood) and juvenile survival (proportion of eggs reaching adulthood) were subjected to Kaplan–Meier survival analysis using the LIFETEST procedure (SAS Institute 2008) to identify the overall effect, and log-rank tests were used for planned pairwise comparisons (Hosmer and Lemeshow 1999). The fertility, pre-oviposition, oviposition, and post-oviposition periods were analysed using the Kruskal–Wallis test employing the NPAR1WAY procedure (SAS Institute 2008). The survival data of the adults were used to construct time-mortality curves using Kaplan–Meier estimators, employing the LIFETEST procedure (SAS Institute 2008), and log-rank tests were used for planned pairwise comparisons. Using the developmental time, viability and female oviposition data, lifetables were constructed and analysed for each treatment as specified in the experiments described in subsection "Effects of acaricides on biological parameters of the treated unmated females (F0)".

Effects of acaricides on the offspring (F2) of F1 females of *Neoseiulus baraki*

The eggs laid by the F1 females (Bioassay: "Effect of acaricides on the developmental and reproductive performance of the offspring of treated females") were collected daily to determine and compare the instantaneous rate of increase (r_i) of the following generation (F2). Every egg was transferred to a new untreated well, placed in a rearing chamber and maintained at 27 ± 1 °C and 70 ± 10 % RH and a 12 h daily photoperiod until they reached adulthood. Coconut mites were provided as food on a piece of perianth (approximately 1 cm^2) containing nearly 300 individuals. When the females emerged, one male was added to each well. Fifteen replicates were performed for each acaricide, and every female represented one replicate. The numbers of eggs, larvae, nymphs and adults were recorded for 10 days. The instantaneous rate of increase (r_i) was estimated using the following equation: $r_i = \ln^{(N_f/N_0)} / \Delta t$, where N_f is the final number of live mites, N_0 is the initial number of live mites and Δt is the time interval between the start and end of the bioassay (Stark et al. 1997; Walthall and Stark 1997), which was carried out for 10 days. Positive r_i values indicate that the population is growing, $r_i = 0$ indicates that the population is stable, and negative r_i values indicate that the population is in decline. The instantaneous rate of increase was analysed using the Kruskal–Wallis test employing the NPAR1WAY procedure (SAS Institute 2008).

Results

Effects of acaricides on the biological parameters of the treated unmated females (F0)

The exposure of *N. baraki* females to the acaricides did not affect the pre-oviposition period ($\chi^2 \geq 0.17$; $df = 1$; $P \geq 0.06$). However, significant differences were observed for the oviposition ($\chi^2 = 28.10$; $df = 3$; $P < 0.001$) and post-oviposition periods ($\chi^2 = 18.11$; $df = 3$; $P < 0.001$). Both periods (oviposition and post-oviposition) were shorter when the females were treated with abamectin. In addition, this product was the only one that

affected the number of eggs per female ($\chi^2 = 28.15$; $df = 3$; $P < 0.001$), which decreased by 96 % (Table 1).

Abamectin was the only acaricide that caused changes in the survival curves ($\chi^2 = 134.32$; $df = 3$; $P < 0.001$) and consequently decreased the median survival time ($\chi^2 = 27.15$; $df = 3$; $P < 0.001$) of *N. baraki* (Fig. 1a, b). This pesticide reduced survival by 76.7 % and the median survival time by 84 %. Adult *N. baraki* did not survive longer than 7 days after exposure to abamectin, and the median survival time was 3.5 ± 0.4 days.

The R_0 was negatively affected by abamectin and was reduced by 96.52 %. Although the other parameters were not affected when *N. baraki* was exposed to abamectin, these parameters were consistently (numerically) lower, and r_m and DT were negative. The values of r_m, T and DT did not differ among the treatments (Table 2). The number of female offspring of *N. baraki* was consistently higher than the number of male offspring, except when females were exposed to abamectin, in which case the only two viable eggs produced males (Table 3).

Effects of acaricides on the development and reproductive performance of the offspring (F1) of treated females

It was not possible to analyse the developmental time, juvenile survival or life-table parameters of offspring of abamectin-treated females of *N. baraki* because only two F0 eggs were viable, and these eggs produced males. For the other acaricides, there was no effect of the exposed *N. baraki* females on the developmental rate of their offspring (Fig. 2; $\chi^2 = 0.66$, $df = 2$, $P = 0.72$), but there was a significant effect on juvenile survival (Fig. 2; $\chi^2 = 6.84$, $df = 2$, $P = 0.03$). This was caused by the lower survival of juveniles from fenpyroximate-exposed females compared with unexposed (control) or azadirachtin-exposed females (Fig. 2; $\chi^2 > 4.38$, $df = 1$, $P < 0.04$).

The survival curves of the offspring of acaricide-exposed females were not significantly different from those of the offspring of unexposed females ($\chi^2 = 1.40$; $df = 2$; $P = 0.49$). There were significant acaricide effects on the R_0, r_m and DT of the offspring of the exposed *N. baraki* females compared to those of the offspring of unexposed females (Table 4). Fenpyroximate was the only acaricide that significantly reduced the R_0 and r_m of the offspring compared with the offspring of azadirachtin-treated and untreated females. As a consequence, the offspring of females exposed to fenpyroximate had a higher DT. There was no effect of acaricide exposure on the generation time (T) of *N. baraki* females compared to those of the offspring of unexposed females.

Table 1 Effects of sublethal exposure of acaricides on the mean (±SE) reproductive performance and female longevity of *Neoseiulus baraki*

Parameters	Treatments			
	Control	Abamectin	Azadirachtin	Fenpyroximate
Period of pre-oviposition (days)	1.3 ± 0.16a	2.9 ± 0.38a	4.9 ± 1.84a	2.0 ± 0.29a
Period of oviposition (days)	15.5 ± 1.7a	0.6 ± 0.16b	11.6 ± 0.16a	13.5 ± 0.16a
Period of post-oviposition (days)	1.7 ± 0.90a	0.1 ± 0.16b	1.7 ± 0.16a	2.3 ± 0.16a
Total number of eggs/females	24.1 ± 2.71a	1.0 ± 0.64b	20.4 ± 3.23a	22.9 ± 4.10a

Means within a row followed by different letters are significantly different (Kruskal–Wallis tests: $P < 0.05$)

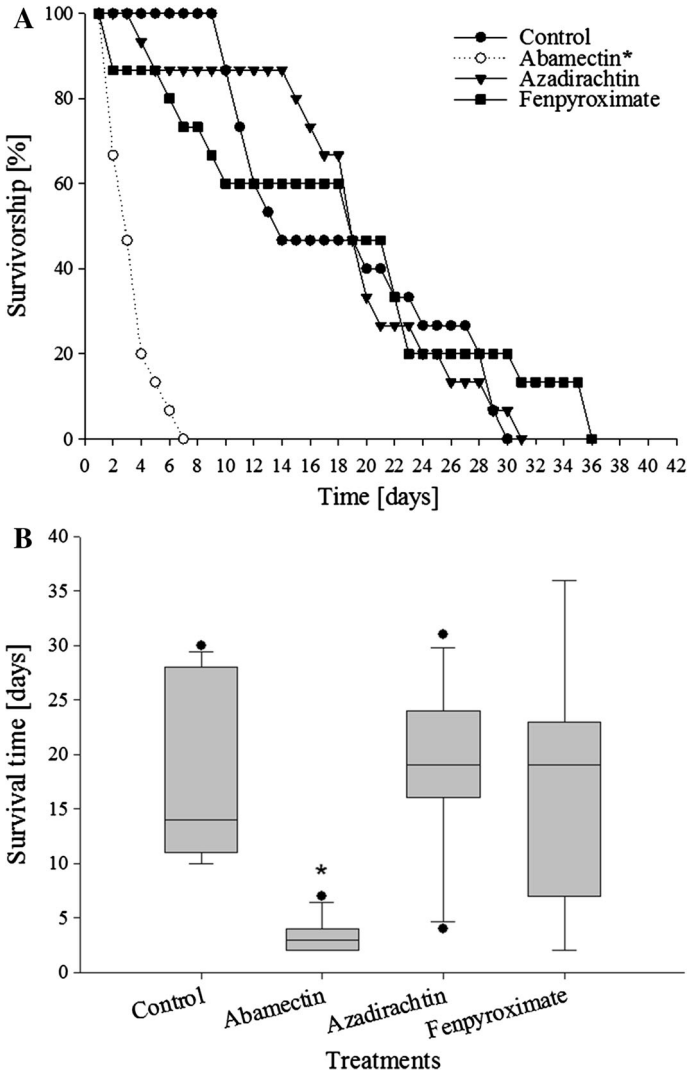

Fig. 1 **a** Survival curves of *Neoseiulus baraki* and **b** respective median survival times when exposed to abamectin, azadirachtin and fenpyroximate. The *asterisk* indicates a significant difference between acaricide-exposed and unexposed predators (log-rank test: $P < 0.05$). The *bottom* and *top* of the boxes are the lower and upper quartiles, respectively, and the horizontal line near the *middle* of the box is the median; the *whiskers* represent the lowest and highest data points within an interquartile range of 1.5 from the first and third quartiles, respectively. Outliers are represented as *dots*

Effects of acaricides on the second generation of exposed *Neoseiulus baraki*

The r_i value of the second generation of the acaricide-exposed females was not significantly different from the control ($\chi^2 = 4.70$; $df = 2$; $P = 0.09$). The r_i values were as follows: control $= 0.22 \pm 0.01$, azadirachtin $= 0.20 \pm 0.01$ and fenpyroximate $= 0.21 \pm 0.01$.

Table 2 Mean life table parameters (+95 % confidence intervals) of *Neoseiulus baraki* treated or not with acaricides at 27 ± 1 °C and 70 ± 10 % RH under a 12 h daily photoperiod

Treatments	R_0 (females/female)	r_m (females/female/days)	T (days)	DT (days)
Control	13.21 (9.33–16.48)	0.20 (0.18–0.22)	12.76 (11.02–14.50)	3.42 (3.10–3.73)
Abamectin	0.46 (−0.17 to 1.10)*	−0.02 (−0.25 to 0.20)	11.74 (7.82–15.65)	−6.90 (−21.56–7.76)
Azadirachtin	9.19 (6.16–12.21)	0.18 (0.15–0.20)	12.63 (11.08–14.19)	3.91 (3.27–4.55)
Fenpyroximate	11.23 (7.19–15.27)	0.18 (0.16–0.21)	13.39 (11.79–15.00)	3.81 (3.26–4.35)

The asterisk indicates a significant difference between the treatment and control based on non-overlapping 95 % CIs after estimating errors using the jackknife method

Table 3 Effects of sublethal exposure of acaricides on the sex ratio of *Neoseiulus baraki* tested by the χ^2 goodness-of-fit test to a 1:1 (female:male) ratio*

Treatments	Observed frequency		Expected frequency		*df*	χ^2	P	Female/(male + female)
	Female	Male	Female	Male				
Control	15	4	9.5	9.5	1	6.36	0.01	0.78
Abamectin	0	2	1.0	1.0	–	–	–	–
Azadirachtin	15	2	8.5	8.5	1	9.94	0.002	0.88
Fenpyroximate	15	6	10.5	10.5	1	3.86	0.049	0.71

* The real sex ratio for *N. baraki* is 0.80 [Female/(male + female)] (Domingos et al. 2010)

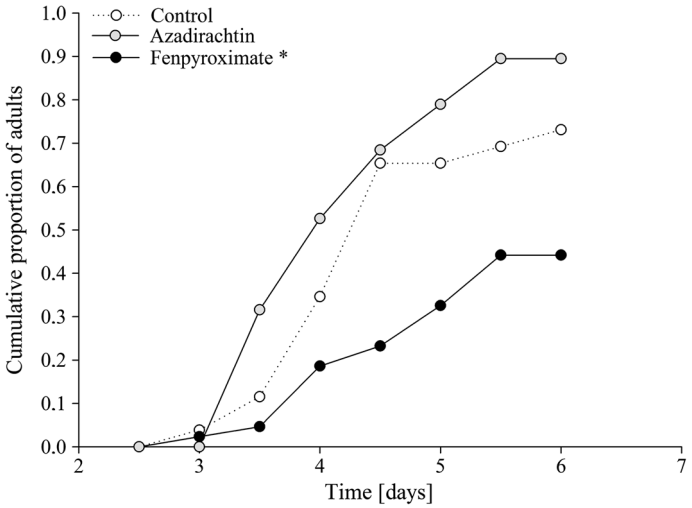

Fig. 2 Proportion of offspring of *Neoseiulus baraki* developing to adults after female exposure to acaricides (azadirachtin and fenpyroximate) or water (control). There were no significant differences in offspring developmental time (days). The *asterisk* indicates a significant difference in the cumulative proportion of adults (log-rank test: $P < 0.05$)

Discussion

Three acaricides registered at the Ministry of Agriculture and widely used against the coconut mite in Brazil (Agrofit 2015) were evaluated with regard to their effects on the predator *N. baraki* upon realistic exposure via contact with contaminated surfaces and ingestion of contaminated prey to identify which compounds are likely to be compatible with biological control of the coconut mite, exhibiting little or no negative impact on the biocontrol agent. The results of our research showed negative effects of abamectin on the oviposition, post-oviposition, survival curves and R_0 of female *N. baraki* after exposure. Reductions in R_0 and r_m as well as an increase in DT were observed in the offspring of fenpyroximate-exposed *N. baraki* females. No biological parameter of *N. baraki* or their offspring was affected by azadirachtin.

Abamectin decreased the oviposition of *N. baraki* as well as its fertility. The adverse effect of abamectin on the fecundity of phytoseiid predators has been demonstrated in

Table 4 Mean (±SE) life table parameters (+95 % confidence intervals) for the offspring of *Neoseiulus baraki* females treated or not with acaricides at 27 ± 1 °C and 70 ± 10 % RH under a 12 h daily photoperiod

Treatments	R_0 (females/female)	r_m (females/female/days)	T (days)	DT (days)
Control	10.05 ± 3.01 (7.03–13.06)	0.20 ± 2.11 (0.16–0.24)	11.38 ± 2.11 (9.27–13.50)	3.39 ± 0.72 (2.68–4.11)
Azadirachtin	15.30 ± 5.75 (9.55–21.05)	0.24 ± 0.03 (0.21–0.27)	11.58 ± 1.63 (9.95–13.21)	2.92 ± 0.37 (2.55–3.29)
Fenpyroximate	3.47 ± 1.71 (1.76–5.17)*	0.11 ± 0.04 (0.07–0.15)*	11.50 ± 1.90 (9.95–13.21)	6.00 ± 2.26 (3.84–8.35)*

The asterisks indicate significant differences between the treatment and control according to non-overlapping 95 % CIs after estimating errors using the jackknife method

several studies (Zhang and Sanderson 1990; Ibrahim and Yee 2000; Bostanian and Akalach 2006; Nadimi et al. 2009; Lima et al. 2013b). Lima et al. (2015a) observed that abamectin exposure can compromise the consumption of *A. guerreronis* by *N. baraki*. A reduction in predator feeding may compromise its fitness. Our results also showed that the population growth rate of the offspring of abamectin-exposed females was significantly reduced. This product has been considered non-selective based on a comparison of its toxicity on *N. baraki* and coconut mite (Lima et al. 2013b).

Although fenpyroximate did not show negative effects on treated females (F0), this product interfered with the offspring of the treated females (R_0, r_m and DT). Our findings agree with those of Hamedi et al. (2010), who reported a decreased oviposition period of fenpyroximate-treated females of the predatory mite species *Phytoseius plumifer* (Canestrini & Fanzago) (Acari: Phytoseiidae), compromising its fertility and that of the subsequent generation. How the acaricides interfere with the offspring of predatory mites is unclear. However, fenpyroximate did not affect the 2nd generation. These results suggest that the impact of fenpyroximate is minimized over the generations. In our study, the predator was exposed only once to the acaricides, which we assumed to be a realistic condition because *N. baraki* lives under the bracts and is not in contact with acaricides that do not reach this area. Nevertheless, field acaricide application is performed at short intervals (at least two times per month) (Melo et al. 2012). Thus, the effects observed here will be more persistent in the field.

Azadirachtin also does not appear to affect the biological parameters of *N. baraki* through subsequent generations (e.g., life table parameters for the first generation and r_i for the second generation). However, Lima et al. (2013a, 2015b, 2016 found changes in the behaviour of *N. baraki* (walking behaviour, overall predator activity, mating behaviour and prey location) when exposed to azadirachtin. These authors suggested that doubts remain regarding the alleged environmental safety and selectivity of this bioinsecticide towards biological control agents.

The results obtained in this study indicate significant negative effects of abamectin and fenpyroximate on *N. baraki*. The use of abamectin and fenpyroximate resulted in adverse consequences for the biological control of *A. guerreronis* using *N. baraki* because both acaricides decreased predator population growth, extending to the 1st but not the 2nd generation of exposed mites. Curiously, azadirachtin did not exhibit significant negative effects on the predator population, which suggests its potential compatibility with biological control for the management of coconut mites. However, field experiments need to be performed using periodic acaricide applications to account for this pattern of pesticide use and to allow for possible predator behavioural changes with exposure, which has been reported elsewhere (Lima et al. 2013a, 2015b) and may result in significant negative impacts on *N. baraki*.

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