Research Article

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Effects of azadirachtin on *Tetranychus urticae* (Acari: Tetranychidae) and its compatibility with predatory mites (Acari: Phytoseiidae) on strawberry

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Abstract

BACKGROUND: The spider mite, *Tetranychus urticae*, is the major strawberry pest in Brazil. The main strategies for its control comprise synthetic acaricides and predatory mites. The recent register of a commercial formula of azadirachtin (Azamax® 12 g L^{-1}) can be viable for control of *T. urticae*. In this work, the effects of azadirachtin on *T. urticae* and its compatibility with predatory mites *Neoseiulus californicus* and *Phytoseiulus macropilis* in the strawberry crop were evaluated.

RESULTS: Azadirachtin was efficient against *T. urticae*, with a mortality rate similar to that of abamectin. In addition, the azadirachtin showed lower biological persistence (7 days) than abamectin (21 days). Azadirachtin did not cause significant mortality of adult predatory mites (*N. californicus* and *P. macropilis*), but it did reduce fecundity by 50%. However, egg viability of the azadirachtin treatments was similar to that of the control (>80% viability). The use of azadirachtin and predatory mites is a valuable tool for controlling *T. urticae* in strawberry crop.

CONCLUSIONS: Azadirachtin provided effective control of *T. urticae* and is compatible with the predatory mites *N. californicus* and *P. macropilis*. It is an excellent tool to be incorporated into integrated pest management for strawberry crop in Brazil.

Keywords: two-spotted spider mite; azadirachtin; *Neoseiulus californicus*; *Phytoseiulus macropilis*; Integrated Pest Management; strawberry

1 INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* (Koch, 1836) (Acari: Tetranychidae), is the main strawberry pest in Brazil and all over the world.[1] This species injures plants, causing premature cell death, premature fall of leaves, production losses and plant death.[2,3] Owing to its high biotic potential, it can quickly inflict economic damage, causing great reductions in the quality and quantity of fruit.[4]

Chemical control is the most common strategy for managing spider mite in the strawberry crop in Brazil. However, the intensive use of acaricides has been compromising the effectiveness of the chemicals, in particular through the development of resistance in several countries,[5–7] including Brazil.[8] Another problem with the use of acaricides is the residue on the fruit. Harvesting of the strawberry is performed daily, and many acaricides have a high residual effect. In addition, the most commonly used acaricides deleteriously affect the predatory mites *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) and *Phytoseiulus macropilis* (Banks) (Acari: Phytoseiidae), which are the main predators of *T. urticae* in Brazil.[9,10] In general, the effects of acaricides on predatory mites comprise mortality of eggs, nymphs and adults, lower prey consumption and reproductive capacity, egg viability decrease and change in sex ratio.[11]

One of the strategies for spider mite control in strawberry is the continuous use of biological control, especially with the predatory mites *N. californicus* and *P. macropilis*.[12] Efficiency of these predators, which depends on the pest population level, is

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seen in low infestations (3–6 spider mites per strawberry leaflet).[4] Several studies have indicated that the predators alone may not be able to maintain spider mite populations below an economic injury level for an extended period of time.[12–14] Thus, selective insecticides/acaricides are needed to adjust the prey/predator ratio and to maintain adequate long-term control efficacy.

Another strategy for control of mite on strawberry crop to reduce the use of synthetic acaricides is the use of plant extracts, especially neem (*Azadirachta indica* A. Juss).[15] The main advantages of using neem are its insecticide and acaricide activity, its low toxicity towards mammals and birds and fast product degradation in the soil and animals,[16] making its use also acceptable in organic productions in Brazil.[17] In 2009, a commercial formula based on azadirachtin A and B (Azamax® 12 g L^{-1}) (DVA Brasil, Campinas-SP, www.dvabrasil.com.br) was registered in the Brazilian market to control pests in agriculture.[18] When compared with old products based on neem, this new formulation has a standardised type and amount of azadirachtin (active ingredient). This formula is authorised for the strawberry crop and it is certified by the Biodynamic Institute (IBD) to be used in organic production without a preharvest interval (PHI). This allows the product to be used at harvesting time without the risk of leaving toxic residues on the final product.

Therefore, strawberry mite management decisions must be planned in order to combine methods of chemical and biological control in a correct, safe and economically feasible form for pest control. In view of the possibility of using azadirachtin and predatory mites for managing spider mites in the strawberry crop, this work aimed to evaluate the effects of azadirachtin on *T. urticae* and its compatibility with the predatory mites *N. californicus* and *P. macropilis*.

2 MATERIALS AND METHODS

2.1 Origin and rearing of *T. urticae*

T. urticae nymphs and adults were collected in October 2009 from strawberry leaves of the 'Aromas' cultivar in a commercial area in Bom Princípio, Rio Grande do Sul State, Brazil (29° 04′ 06″ S, 51° 22′ 40″ W). The mites were reared on strawberry plants of the 'Festival' cultivar in a greenhouse and kept in 3 L buckets filled with the substratum.

2.2 Effects of azadirachtin against *T. urticae*

The study was conducted in a greenhouse at Embrapa Grape & Wine, Bento Gonçalves, Rio Grande do Sul State, Brazil. For the experiment, strawberry plants of the 'Aromas' cultivar (3 months old) were used, with ≈5 leaves $plant^{-1}$. The infestation was made per plant individually, using 30–40 individuals $leaf^{-1}$ (nymphs and adults) of *T. urticae*. Three days after infestation, one strawberry leaf of each infested plant was marked on the peduncle area using a strip of white cloth humidified with petroleum jelly to prevent the escape of the mites at application time of the products and to allow evaluation of the experiment. The dryness of the petroleum jelly was checked daily, and, whenever necessary, it was replaced with the help of a brush. Application was carried out 3 days after infestation, with a spray volume of 800 L water ha^{-1}, using a manual back device model 'Jacto' PJT Teejet XR11008VS of 20 L capacity. The experimental design was completely randomised with five repetitions, each composed of four strawberry leaves, with a total of 20 plants per treatment. In the control, plants were sprayed with water only. The evaluated treatments were abamectin (Vertimec 18 CE® at 18 mL AI 100 L^{-1} water), azadirachtin (Azamax® 12 g L^{-1} at 1.2, 2.4 and 3.6 mL AI 100 L^{-1} water) and one control (water). All concentrations of azadirachtin were reapplied 7 days after the first application. Before the first application, pre-sampling was carried out by counting the number of *T. urticae* per leaf. After application, the number of survivors was recorded by counting the number of mites per leaf at 1, 7 and 15 days after the first application. The percentage of population reduction in the treatments was corrected in relation to the control (water) by Henderson and Tilton's formula.[19] Afterwards, the data were submitted to the Shapiro–Wilk normality test (PROC UNIVARIATE).[20] All population reduction data were transformed into $\sqrt{x + 0.5}$ and submitted to repeated-measurement analysis for interaction evaluation of explanatory variables (treatments, dose and time), and the means were compared by the Tukey–Kramer test ($P < 0.05$) (PROC GLM).[20]

2.3 Biological persistence of azadirachtin against *T. urticae*

To evaluate the biological persistence of azadirachtin against *T. urticae*, the same treatments as those described above were sprayed once only over 20 strawberry plants of the 'Aromas' cultivar kept in a greenhouse. The plants were not infested with *T. urticae*. At 1, 3, 5, 7, 10, 15, 21 and 28 days after application, one leaflet of the middle region of each plant was removed and taken to the laboratory (temperature 25 ± 1 °C, relative humidity 70 ± 10%, 12 h photophase). The leaves (adaxial surface down) were placed under a layer of agar water (3%), using one leaflet per petri dish (1.3 cm height × 6.5 cm diameter). The experimental design was entirely randomised, with ten repetitions per treatment. Every repetition (arena) was infested with ten adults. After the infestations, the dishes were placed in a climatic chamber (temperature 25 ± 1 °C, relative humidity 70 ± 10%, 12 h photophase). The mortality was recorded under a stereomicroscope at 24 h after the leaflets were infested. A spider mite was considered dead if no perceptible movement occurred after it was touched with a fine brush. The data were corrected and analysed as previously described.

2.4 Effect of azadirachtin on *N. californicus* and *P. macropilis*

The predatory mites *N. californicus* and *P. macropilis* were obtained from PROMIP Ltda and reared on bean leaves (*Canavalia ensiformis*) and fed with *T. urticae*. The experiments were conducted in the laboratory with predatory mites that were ≈7 days old. Each predatory mite was evaluated separately. The experiments were carried in petri dishes (1.3 cm × 6.5 cm) by placing ten adults of *N. californicus* or *P. macropilis* at a ratio of 4 females to 1 male per petri dish containing one strawberry leaflet of the 'Aromas' cultivar under an agar water layer (3%). A strip of hydrophilic cotton was placed on the leaflet borders to prevent the escape of predatory mites, forming an arena. The predatory mites were transferred to the strawberry leaflet with a fine tip brush. Next, the dishes containing strawberry leaves (arena) and the mites were sprayed in a Potter spray tower (Burkard Manufacturing, Rickmansworth, Herts, UK) from a 20 cm distance at 10 lb in^{-2} pressure, resulting in a spray deposition of 1.7 mg cm^{-2}. The products evaluated were the same as in the greenhouse experiments (Section 2.2). Thirty minutes after application, 200 *T. urticae* were placed in each arena, resulting in an average of 20 spider mites per predatory mite to serve as a feeding substratum. Every 48 h, the spider mites were replaced. The factorial experimental design was 5 × 2 (treatments × predatory mites), with the five treatments consisting of one concentration of abamectin (Vertimec 18 CE® at 18 mL AI 100 L^{-1}

Table 1. Population reduction (mortality) of *T. urticae* after azadirachtin and abamectin applications on strawberry leaves in laboratory trials

	Dose (mL 100 L^{-1} water)		Days after first application[a]						
			Pre-sampling[d]	1		7[e]		15	
Active ingredient	AI[b]	CP[c]	*n*	*n*	%M[f]	*n*	%M	*n*	%M
Azadirachtin	1.2	100	37.0 ± 3.01 a	34.5 ± 2.94 b	20	15.0 ± 1.20 b	72	3.3 ± 0.22 a	94
Azadirachtin	2.4	200	40.5 ± 4.28 a	34.0 ± 4.56 b	40	13.0 ± 4.10 b	78	2.0 ± 0.72 a	97
Azadirachtin	3.6	300	32.7 ± 6.86 a	27.0 ± 6.07 ab	31	10.0 ± 1.41 b	79	0.0 ± 0.00 a	100
Abamectin	18	75	31.0 ± 1.17 a	15.5 ± 1.89 a	60	1.5 ± 0.52 a	97	1.5 ± 0.60 a	97
Control (water)	–	–	31.8 ± 4.40 a	38.0 ± 4.56 b	–	47.0 ± 2.26 c	–	51.0 ± 2.89 b	–

[a] Values represent means ± SE. Means followed by the same letter in a column are not significantly different for the performance measurement (Tukey–Kramer test, $P < 0.05$).
[b] AI: active ingredient.
[c] CP: commercial product.
[d] First application of azadirachtin.
[e] Second application of azadirachtin.
[f] %M: mortality corrected by Henderson and Tilton's formula.[19]

water), three concentrations of azadirachtin (Azamax® 12 g L^{-1} at 1.2, 2.4 and 3.6 mL AI 100 L^{-1} water) and one control (water), and with the two species of predatory mites *N. californicus* and *P. macropilis*, for a total of ten dishes per treatment per species. The dishes containing strawberry leaflets (arenas) were kept in a climatic chamber (temperature 25 ± 1 °C, relative humidity 70 ± 10%, 12 h photophase). The survival of predatory mites was evaluated at 24, 48, 72 and 96 h after application (HAA) under a stereomicroscope. Predatory mites were considered dead if they did not move for a distance equivalent to their body length after touched with a fine tip brush. The fecundity of females was recorded 72 HAA by counting the number of eggs deposited in each arena, and was expressed in eggs female^{-1} day^{-1}. The eggs were then transferred to a new leaflet of a strawberry of the same cultivar (free of product contamination) for viability evaluation (number of viable eggs). The viability of eggs of predatory mites was recorded for 8 days without changing the strawberry leaflet. The mortality data in the azadirachtin and abamectin treatments were corrected in relation to the control (water) by Abbott's formula.[21] All data were submitted to the Shapiro–Wilk normality test (PROC UNIVARIATE).[20] Thereafter, all data were transformed into $\sqrt{x + 0.1}$ and submitted to analysis of variance, and the means were compared by Tukey's test ($P \leq 0.05$) (PROC ANOVA).[20] The mortality and fecundity data were analysed separately for each species and then together.

3 RESULTS

3.1 Effect of azadirachtin against *T. urticae*

There were no interactions between the explanatory variables (treatment, dose and time) because they are independent. This indicates that the response (population reduction of *T. urticae*) depends only on the acaricide dose, regardless of time of evaluation ($F = 9.77$; df = 4, 95; $P = 0.5914$).

The spider mite, *T. urticae*, was susceptible to different doses of azadirachtin (Azamax 12 g L^{-1}) evaluated. The azadirachtin at 1 day after first application (DAFA) at doses of 1.2, 2.4 and 3.6 mL AI 100 L^{-1} water caused a population reduction ranging from 20 to 40% (Table 1). Another important finding was that azadirachtin did not show a direct population reduction response to dose increase. In this analysis, abamectin at 18 mL AI 100 L^{-1} water was significantly more efficient against *T. urticae* (60% population reduction) compared with azadirachtin (40% population reduction maximum) ($F = 5.71$; df = 4, 95; $P = 0.0004$).

At 7 DAFA, azadirachtin at different doses caused a population reduction ranging from 72 to 79%, while abamectin reduced infestation by 97% (Table 1). Similarly to the evaluation at 1 DAFA, the population reduction caused by azadirachtin at 7 DAFA was significantly lower than that caused by abamectin ($F = 66.18$; df = 4, 95; $P < 0.0001$), and increase in the azadirachtin dose did not affect the population reduction.

In the final evaluation, at 15 DAFA or 7 days after the second application, the population reduction at different azadirachtin doses ranged from 94 to 100% (Table 1). In this evaluation, azadirachtin caused a high population reduction of *T. urticae*, which did not differ statistically from the reduction caused by abamectin acaricide, but both treatments differed from the control ($F = 137.53$; df = 4, 95; $P < 0.0001$) (Table 1).

3.2 Biological persistence of azadirachtin against *T. urticae*

Similarly to the previous experiment, there were no interactions between the variables (treatment, dose and time) in the biological persistence study ($F = 9.03$; df = 4, 95; $P = 0.6605$). The biological persistence evaluated at 1 day after application (DAA) of three azadirachtin doses showed a population reduction ranging from 20 to 40%, and it is statistically lower than that of abamectin (≈60% population reduction) ($F = 119.44$; df = 4, 45; $P < 0.0001$) (Fig. 1).

At 3 DAA there was an increase in the biological activity of azadirachtin (40–64% population reduction), differing from the control and abamectin treatments ($F = 218.01$; df = 4, 45; $P < 0.0001$) (Fig. 1). The same results were observed at 5 DAA ($F = 276.01$; df = 4, 45; $P < 0.0001$). At 7 DAA it was observed that azadirachtin, at all concentrations evaluated, presented a decrease in biological activity (population reduction <60 %), differing from abamectin (≈95% population reduction) and control treatments ($F = 344.06$; df = 4, 45; $P < 0.0001$) (Fig. 1).

Evaluations at 15, 21 and 28 DAA revealed a continuous population reduction of spider mites exposed to different azadirachtin doses (Fig. 1). This reinforces the need to reapply

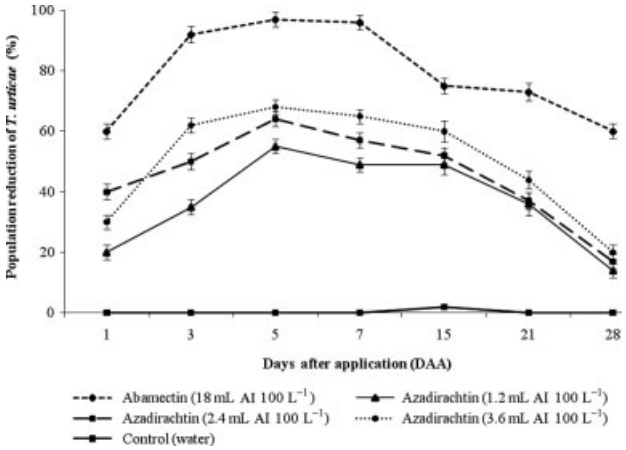

Figure 1. Biological persistence of azadirachtin and abamectin against *T. urticae* on strawberry plants in greenhouse trials. Values represent means ± SE after correction by Henderson and Tilton's formula.[19].

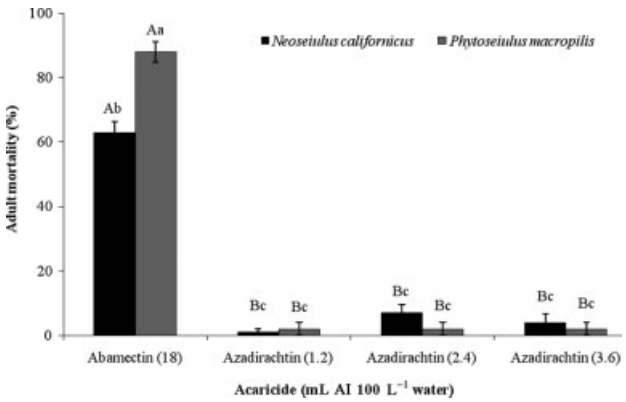

Figure 2. Adult mortality of *N. californicus* and *P. macropilis* 96 h after azadirachtin and abamectin application in laboratory trials. Values represent means ± SE after correction by Abbott's formula. Values with the same upper-case letter do not differ for individuals of the same species, and those with the same lower-case letter do not differ for individuals of different species (Tukey's test, $P \leq 0.05$).

azadirachtin 7 days after the first application (peak of biological activity) for effective control *T. urticae*. At 28 DAA, azadirachtin at the three concentrations resulted in a <18% population reduction, differing statistically from abamectin which provided a control of 55% when the experiment was finished ($F = 192.04$; df = 4, 45; $P < 0.0001$) (Fig. 1).

3.3 Effects of azadirachtin on *N. californicus* and *P. macropilis*

The azadirachtin (Azamax 12 g L^{-1}) showed low toxicity to *N. californicus* and *P. macropilis* (Fig. 2). After 72 h of azadirachtin application, the mortality of *N. califonircus* was no different in the three doses evaluated (≈7% mortality); however, there was highly significant mortality in the abamectin treatment (≈60%) ($F = 59.43$; df = 4, 45; $P < 0.0001$). The same results were found for *P. macropilis* (>85% mortality in abamectin treatment) ($F = 117.22$; df = 4, 45; $P < 0.0001$). In addition, the mortality of predatory mites was statistically lower in the azadirachtin doses than in the abamectin doses ($F = 77.03$; df = 4, 45; $P < 0.0001$).

On the other hand, female survivors of *N. californicus* ($F = 24.99$; df = 4, 45; $P < 0.0001$) and *P. macropilis* ($F = 54.77$; df = 4, 45; $P < 0.0001$) at the three doses of azadirachtin showed a reduction

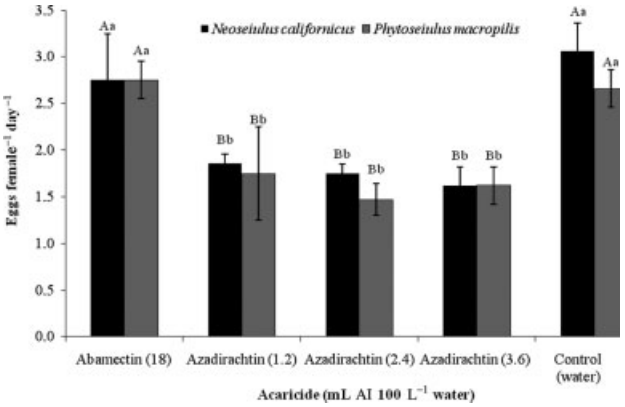

Figure 3. Fecundity (eggs female^{-1} day^{-1}) of *N. californicus* and *P. macropilis* 72 h after azadirachtin and abamectin applications in laboratory trials. Values represent means ± SE. Values with the same upper-case letter do not differ for individuals of the same species, and those with the same lower-case letter do not differ for individuals of different species (Tukey's test, $P \leq 0.05$).

in daily fecundity (eggs female^{-1} day^{-1}) (≈50%) for both species, differing statistically from the individuals under the abamectin treatment (Fig. 3). However, no statistically significant differences were observed in egg viability (>80%) of *N. californicus* ($F = 9.01$; df = 4, 45; $P < 0.5674$) and *P. macropilis* ($F = 7.03$; df = 4, 45; $P = 0.6909$) in the treatments.

4 DISCUSSION

The azadirachtin (Azamax 12 g L^{-1}) was efficient in population reduction of *T. urticae*. The control provided by azadirachtin in this study was similar to that provided by other neem formulations against *T. urticae*, e.g. NeemAzal® at 0.4% and Oikos® at 4.5%, which, at 72 HAA, caused mortality ranging from 85 to 100%.[22–25] However, the neem-based product Natuneem® at 0.25 and 1.0% caused unsatisfactory mortality (40–56%) of *T. urticae* at 72 HAA when applied on bean leaves (*C. ensiformis*).[23] In addition to mortality, sublethal doses of azadiracthin had a negative effect on longevity, fecundity and life table parameters of *T. urticae*.[26]

The neem-based products also provided control of other spider mites. Neem-I-Go® at 0.5 and 2% showed toxic effects to *Brevipalpus phoenicis* (Acari: Tenuipalpidae) on citrus and to *Polygotarsonemus latus* (Banks) (Acari: Tarsonemidae) on chilli pepper.[27,28] This last result is particularly important, because *P. latus* is another spider mite that infests strawberry crop in Brazil and for which azadirachtin can be an effective control. However, several studies have demonstrated a slower effect of some neem-based products when applied on *T. urticae* in comparison with synthetic acaricides.[29,30] *T. urticae* has high rates of reproduction and may be able to overcome the effects of a pesticide because survivors produce more offspring by comparison with species with low reproductive potential.[31] In this case, reapplications of azadirachtin might increase the control or reduce the population. This was observed in the present study, in which, because of the high biotic potential of *T. urticae* in the strawberry crop, the level of control obtained in all azadirachtin concentrations 7 DAA was considered unsatisfactory (<80%). Therefore, a new application of azadirachtin was necessary to obtain a high control efficacy similar to that achieved with abamectin.

The biological persistence of azadirachtin showed a significant population reduction of *T. urticae* until 5 DAA. Normally, the highest

peaks of neem-derived products that translocate in the plant occur at 5 DAA, and they are stored in the roots, stems and leaves of plants up to a maximum of 8 days.[32] Afterwards, the effectiveness of the products declines. The decrease in biological activity of azadirachtin can be attributed to the effects of temperature and ultraviolet light, which cause product degradation in the plant. Several studies showed that temperature, luminosity and rainfall are the main factors contributing to neem degradation.[33–35] For this study, rainfall was not a factor of degradation, because the strawberry plants were kept in greenhouses. These aspects reinforce the hypothesis that 7 days is an ideal time to reapply azadirachtin for the effective control of *T. urticae* in strawberry crop.

On the other hand, abamectin showed a population reduction of *T. urticae* for ≈21 DAA. The long residual time of abamectin could be perceived as an advantage in terms of spider mite control. However, it is toxic to *N. californicus* and *P. macropilis*, the main biological control agents of *T. urticae* in strawberries in Brazil.[9] Toxicity of abamectin towards the predatory mites *N. californicus* and *P. macropilis* has also been reported in several other studies.[3,5,7]

Another important characteristic of azadirachtin was its compatibility with *N. californicus* and *P. macropilis*. Studies showed the compatibility of neem-based products with predatory mites, e.g. azadirachtin (Triact® 70 EC and Oikos® at 4.5%) was compatible with *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) because they are active for only a short period.[24,29,36] However, the effects of neem-derived compounds on predatory mites can vary, depending on the formulation or on the part of the plant used. In a selectivity test of adult *Iphiseiodes zuluagai* Denmark & Muma (Acari: Phytoseiidae), a predatory mite mortality of ≈88% was observed when neem cake was applied. Neem cake presents higher toxicity because 90% of the azadirachtin is concentrated in this compound as the seed is pressed to obtain neem.[37]

In the present study, azadirachtin caused a significant reduction in the fecundity of both predatory mites (*N. californicus* and *P. macropilis*), highlighting the negative effect of azadirachtin on mite fecundity when individuals are in contact with leaves of strawberry containing this substance. For *P. persimilis*, similar results were observed when in contact with bean leaves treated with azadirachtin.[38] However, in a residual toxicity test on strawberry leaves, the neem-based product Oikos® at 4.5% did not negatively affect the fecundity of *N. californicus*.[39] The reduction in fecundity caused by azadirachtin occured owing to failure of germ cells in males and females, which may have contributed to the reduced fecundity of *N. californicus* and *P. macropilis*.[34]

In contrast to fecundity, azadirachtin did not affect egg viability of *N. californicus* and *P. macropilis*. Neem-derived products have often shown higher toxicity to eggs of phytophagous mites than to eggs of predatory mites.[15] The low toxicity of neem-based formulas towards predatory mites can be attributed to the action of enzymes such as esterases, glutathion *S*-transferases and oxidative enzymes, which function in the detoxification of insecticides.[40]

The results indicate that azadirachtin can provide effective control of *T. urticae* and shows low toxicity towards the predatory mites *N. californicus* and *P. macropilis*; both methods could be used separately or in combination for strawberry mite management. Azadirachtin can be used alone because it has two applications (7 day interval) in order to control *T. urticae* at a similar level to abamectin. In addition, the use of azadirachtin is important for the conservation of biological control relying on the preservation of existing natural enemies, which is essential for the control of *T. urticae* in the strawberry crop. This would minimize insecticide/acaricide applications and, consequently, the selection of resistant spider mite populations and fruit contamination.

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