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Systemic activity of azadirachtin on *Oligonychus yothersi* (Acari: Tetranychidae) on yerba mate plants

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ABSTRACT

Aiming to the red mite *Oligonychus yothersi* control in yerba-mate, the systemic action of an azadirachtin-based product (12 g of azadirachtin·L^{-1}) was evaluated in the laboratory. To assess the activity on oviposition and mortality of the mite (1), three weekly applications of the product in an aqueous solution (30, 48, and 66 mg i.a.·L^{-1}) were performed. The applications were carried out in the soil, next to the base of yerba mate seedlings. After seven days of each application, leaves were removed for infestation with 15 adult mite females. To evaluate the activity in the development, survival, and reproduction of the progeny (2), yerba mate seedlings were irrigated with azadirachtin solution of 30 mg i.a.·L^{-1} (recommended concentration). After seven days, leaves were removed and infested with adult females for oviposition and observation of the progeny. Reduction in fecundity (from 23.7 to 44.2%), fertility (27.4 to 48.1%), and an increase in mortality from 14.6 to 47.5% were observed in females fed on azadirachtin-treated plants. Continuous feeding on plants treated with azadirachtin solution significantly reduced oviposition, pre-oviposition period, and longevity of males. In addition, the viability of the egg-adult, and the duration of the egg-adult period increased. The potential of azadirachtin for the control of *O. yothersi* and its action from the irrigation of yerba mate plants was confirmed.

Keywords: *Ilex paraguariensis*; natural acaricide; control.

INTRODUCTION

Yerba mate (*Ilex paraguariensis*) (St Hil.) (Aquifoliaceae) is native to South America, and it occurs naturally in Brazil, Argentina, and Paraguay (CARDOZO JUNIOR; MORAND, 2016), comprising one of the oldest agroforest systems. It has great environmental and socio-economic importance for the states of Rio Grande do Sul and Paraná, Brazil, which are the largest yerba mate producers in the country (IBGE, 2022). Leaves are typically used as raw material for foods, beverages, cosmetics, and in pharmacological studies (OLIVEIRA; WAQUIL, 2015; PIRES et al., 2016; CROGE et al., 2021). Yerba mate is also a rich source of bioactive phenolic compounds, and its uptake is recommended to mitigate some cardiovascular risk factors (CARDOZO JUNIOR; MORAND, 2016). Benefits to human health might vary depending on genetic, environmental, and technological variables, and even on the handling method for production (PIRES et al., 2016; CARDOZO et al., 2021).

Due to increased demand, monoculture exploitation has been growing, which increases leaf supply. However, these areas have biodiversity loss compared to natural areas, and, consequently, they have higher populations of organisms that attain pest *status* (PENTEADO et al., 2000), as observed by BORGES et al. (2003), who found a lower occurrence of pests in native herb gardens than in denser plantations.

Among the mites that attack this crop, the yerba mate red mite, *Oligonychus yothersi* (McGregor) (Acari: Tetranychidae), is one of the most frequently found attacking the adaxial side of yerba mate leaves, causing leaf bronzing and drop when

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there are high infestations (ALVES et al., 2004; FERLA et al., 2005; GOUVEA et al., 2006). A characteristic of the mouthparts of these mites is the presence of a long and retractable cheliceral stylet that pierces the leaf tissue allowing the consumption of cellular contents by suction (ALBERTI; KITAJIMA, 2014; BENSOUSSAN et al., 2016).

In Brazil, the use of chemical pesticides is not allowed for the control of pest organisms on yerba mate crops (AGROFIT, 2022), which explains the search for alternatives. On the other hand, products derived from neem (*Azadirachta indica* A. Juss) (Meliaceae) are used for the control of several agricultural pests. The primary active ingredient of this plant is azadirachtin, which is well known for its repellent and phagoinhibitor properties, and for its interference in the development and reproduction of mites and insects (SCHMUTTERER, 1990; MORDUE; NISBET, 2000; WEATHERSBEE; MCKENZIE, 2005; SCHLESENER et al., 2013; SÁNCHEZ-RAMOS et al., 2014). Neem oil has already been tested against the red mite (PASINI et al., 2003). Its most frequently used form is the oil extracted from neem fruits and seeds. In the market, there are products based on azadirachtin. Azamax is a commercial product based on 1.2% azadirachtin A and B in vegetal oil emulsion.

There are several reports of the use of neem derivatives against insects and mites in pest control, with different application methods in both organic and conventional crops (SCHMUTTERER, 1990; WEATHERSBEE; MCKENZIE, 2005; BERNARDI et al., 2013; SCHLESENER et al., 2013; FUENTES et al., 2019). The activity of Azamax® has been proven in the laboratory aiming to control the red mite in yerba mate. The product in water solution (30 mg i.a.·L^{-1}) was sprayed over yerba mate leaves, having caused approximately 90% of mite mortality, repellency, and ovicidal activity. In the field test, a reduction of mites of 59.6% was observed after spraying the solution on leaves (ALVES et al., 2016).

Aside from spraying, irrigation has also been studied as a method of application of azadirachtin, aiming at the control of pest insects and mites. Previously, it has been proved that azadirachtin has systemic circulation in plants (THOEMING et al., 2006). Also, SUNDARAM et al. (1995) observed a decrease in the population of the mite *Tetranychus urticae* Koch (Acari: Tetranychidae) in poplar plants irrigated with azadirachtin. Changes in the development and mortality of *Aceria guerreronis* Keifer (Acari: Eriophyidae) also occurred in coconut trees treated with azadirachtin in irrigation treatment (SUJATHA et al., 2005; BAGDE et al., 2014; HEGADE et al., 2017).

Considering the successful use of neem in insect control programs and its potential as a pesticide, it is important that its systemic activity for the control of mites is further explored. Although the efficacy of the azadirachtin spraying on yerba mate red mite in the field conditions (ALVES et al., 2016) is known, its systemic effect on the yerba mate plant for the control of *O. yothersi* has not been studied yet. Therefore, the aim of the present study was to evaluate the systemic activity of azadirachtin on *O. yothersi* applied via irrigation on yerba mate plants.

MATERIAL AND METHODS

Bioassays were developed under controlled conditions: 26 ± 1 °C, 60 ± 10% relative humidity (RH), and 12 h of photophase. The commercial product Azamax® (12 g of azadirachtin·L^{-1}, in concentrated oil emulsion) was evaluated using concentrations of 30, 48, or 66 mg a.i.·L^{-1} in distilled water solution, based on the recommendations of the product manufacturer.

Plants

Yerba mate plants approximately 20-cm high were cultivated in plastic containers (700 mL) containing the organic compound (worm humus with earth, charcoal, and grounded pinus bark) in an environment protected by a 50%-polypropylene mesh and with irrigation every two days. Before the beginning of the experiments, leaves were cleaned carefully with cotton moistened with a solution of sodium hypochlorite (1%) and distilled water.

Mite rearing stock

Mites derived from yerba mate plants from the city of Cascavel, Paraná (24°58'05.2"S; 53°24'30.9"W and 24°55'10.3"S; 53°23'15.4"W), were maintained in arenas made of yerba mate plants placed on polyurethane foam moistened with distilled water in Gerbox (11 cm × 11 cm × 3 cm). Each leaf was placed with the adaxial side facing upwards, the edges were enclosed with moistened cotton, and 25 mites were placed on each arena (20 females and five males). Arenas were maintained in rectangular plastic trays at 26 ± 1°C, 60 ± 10% RH, and 12 h of photophase (TOLDI et al., 2016).

Bioassays

Activity on oviposition, fertility, and mortality of adults

A total of 45 yerba mate plants were daily irrigated with 20 mL of distilled water. On the sixth day, irrigation was suspended. One more day later, the plants were divided into four groups of 10 plants. Thus, 70 mL of azadirachtin solutions (30, 48, or 66 mg a.i.·L^{-1} were applied on the soil, near the base of the plants. Control plants received only distilled water. One week after the application, one leaf was removed from each plant to make the arenas (as described before), and each arena received 15 mated adult females from the rearing stock. The arenas were maintained in a rectangular tray (38 × 27 cm), and remained at 26 ± 1°C, 60 ± 10% RH, and 12 h of photophase, throughout the bioassay assessment period. The arenas were evaluated daily, at 1 p.m., for five days to check oviposition and mortality. Those mites who did not react to the touch of a brush were considered dead. After this period, the surviving females were removed from the arenas, and eggs were daily evaluated for more seven days, to assess fertility.

After removing the leaves, the entire leaf maintenance procedure was repeated with daily watering for six days, water fasting for 24 h, and irrigation with the corresponding azadirachtin solutions. Leaf sampling procedure and mite infestation were repeated at 14 and 21 days after application.

Activity on development, survival, and fertility

Another yerba mate plants were divided into two groups with 30 plants. One group was irrigated with azadirachtin 30 mg a.i.·L^{-1}, and the other group received only distilled water (control). For the experiment, one leaf was removed from each plant to mount 30 arenas/treatment. Three adult females from the rearing stock were transferred to these arenas to ovipositing. After 24 h, females were removed, and only one egg/arena remained. Arenas were maintained in rectangular plastic trays of 38 × 27 cm in a germination chamber (26 ± 1°C; 60 ± 10% RH, and 12 h of photophase). The mite development and survival were daily checked at 8 a.m., 1 p.m., and 6 p.m. until they reached the adult phase. In the adult phase, couples were formed, by adding one male from the rearing stock to the arena. In the adult phase, evaluations were performed daily at 1 p.m., checking the number of eggs laid and adult mortality.

Statistical analysis

The normality assumption was tested using Shapiro-Wilk's test ($p < 0.05$) and the homogeneity of variances was tested using Bartlett's test ($p < 0.05$). Data were submitted to a two-way analysis of variance (ANOVA, $p < 0.05$) to determine the difference between treatments, using Statistica, version 7.0.

Tukey's test ($p < 0.05$) was used as a *post hoc* test for multiple comparisons between weeks, between treatments, and between treatments associated to weeks, using GraphPad PRISMA 8.02 (trial version). For mortality correction, the Schneider-Orelli formula was used. Tukey's test ($p < 0.05$) was also used to compare the duration of the egg, larval, protonymph, and deutonymph phases, using Bioestat 5.3.

Data from fertility and duration of the pre-oviposition and oviposition periods, as well as longevity, were submitted to a Student-t test ($p < 0.05$), using Bioestat 5.3.

RESULTS AND DISCUSSION

Activity on oviposition, fertility, and mortality of adults

Azadirachtin had a negative effect on oviposition of *O. yothersi* females (Table 1). At the highest concentration (66 mg a.i.·L^{-1}), oviposition significantly decreased in the third week, from 141 to 84.6 eggs. The same trend was observed at the concentration of 48 mg a.i.·L^{-1}. However, the decrease was already significant from the second week onwards, going from 128.5 to 70.1 eggs. The mean value of oviposition in all the azadirachtin treatments had significantly lower values than the control (Table 1).

The fertility in the first and third weeks of application was significantly lower at the concentration of 30 mg a.i.·L^{-1} (Table 1), as well as from the second week onwards using the concentration of 48 mg a.i.·L^{-1}. In the third week of application, fertility decreased with the three concentrations, with no statistical difference between them. In the control treatment, fertility was not affected. Fertility is an important biological parameter that is affected by azadirachtin (MORDUE; BLACKWELL,

1993). There was no significant difference in fertility as a function of the concentration of azadirachtin used in the treatments. It is possible that the saturation of the product in the plant has occurred, or it is even a reaction of the plant to protect itself from the amount of product received (SUNDARAM et al., 1995).

Table 1. Oviposition and fertility of *Oligonychus yothersi* from on yerba mate leaves after weekly irrigations with azadirachtin A/B 12 g·L^{-1} at concentrations 30, 48, and 66 mg a.i.·L^{-1} in the laboratory (26 ± 1°C, RH = 60 ± 10%, and 12 h of photophase)#.

Concentration (mg a.i.·L^{-1})	Applications				
	1st	2nd	3rd	Mean value	Reduction (%)*
	Oviposition				
66	75.90 ± 14.10 Ba	115.50 ± 22.50 Aa	77.60 ± 5.00 Ba	89.60 ± 12.90 B	44.20
48	128.50 ± 17.90 Aa	70.10 ± 12.50 B	75.40 ± 13.10 Bb	91.30 ± 18.60 B	43.20
30	141.40 ± 20.09 Aa	141.80 ± 12.33 Aa	84.60 ± 9.60 Bb	122.60 ± 19.00 B	23.70
Control	169.10 ± 14.00 Aa	155.80 ± 9.80 Aa	157.20 ± 9.40 Aa	160.70 ± 4.20 A	-
Concentration (mg a.i.·L^{-1})	**Fertility**				
66	129.70 ± 13.80 Aa (91.96)	130.50 ± 7.30 Aa (91.82)	73.00 ± 8.10 Bb (97.17)	111.10 ± 19.00 B	27.35%
48	117.30 ± 18.40 Aa (89.12)	62.20 ± 13.10 Bb (87.02)	65.70 ± 14.40 Bb (85.79)	81.70 ± 17.80 BC	46.54%
30	65.00 ± 11.20 Bb (84.62)	106.00 ± 16.80 Aa (92.60)	66.90 ± 6.11 Bb (85.79)	79.30 ± 13.30 BC	48.13%
Control	157.30 ± 13.20 Aa (93.23)	149.90 ± 9.50 Aa (96.31)	151.50 ± 9.90 Aa (97.17)	152.90 ± 2.20 A	-

#Mean values (± standard error) followed by the same uppercase letter in the column and lowercase letter in the rows do not differ from each other according to Tukey's test ($p < 0.05$). values in parentheses represent reduction (%) in fertility; *compared to the control group; **related to oviposition; RH: relative humidity. Source: Elaborated by the authors.

Mortality was significantly higher in the presence of the concentration of 30 mg a.i.·L^{-1} only in the third week (62.7%). A significant difference was already observable (47.3%) from the second week onwards at the concentration of 48 mg a.i.·L^{-1}. However, there was no significatively relation between mite mortality and the number of applications, at the higher concentration, over the period (Table 2). Regarding the concentrations used, there was no significant difference in the first week compared to the control. However, from the second week onwards, mortality was significatively higher with 48 and 66 mg a.i.·L^{-1} treatments. Finally, after three applications, both 30 and 48 mg a.i.·L^{-1} treatments led to higher mortality, corroborating HEGADE et al. (2017), who tested an azadirachtin-based commercial product applied near the roots of coconut plants and obtained significant results of *A. guerreronis* population decrease along the time.

Table 2. Mortality of adult females of *Oligonychus yothersi* on yerba mate plants after weekly irrigations with Azadirachtin A/B 12 g·L^{-1} at concentrations 30, 48, and 66 mg a.i.·L^{-1} in the laboratory (26 ± 1 °C, RH = 60 ± 10%, and 12 h of photophase)#.

Concentration (ml·L^{-1})	Applications (%)					
	1st	MC%	2nd	MC%	3rd	MC%
66	38.00 ± 1.06 Aa	16.0	48.00 ± 1.12 Aa	24.5	39.30 ± 1.29 Ba	14.6
48	28.00 ± 0.85 Ab	2.4	47.30 ± 1.29 Aa	23.5	49.30 ± 1.79 Aa	28.7
30	38.00 ± 1.56 Ab	15.9	28.70 ± 1.39 Bb	-	62.70 ± 1.03 Aba	47.5
Control	26.20 ± 0.87 Aa	-	31.10 ± 1.09 Bb	-	28.90 ± 0.67 Ba	-

#Mean values (± standard error) followed by the same uppercase letter in the column and lowercase letter in the rows do not differ from each other according to Tukey's test ($p < 0.05$); MC: mortality correct by Schneider-Orelli formula; RH: relative humidity. Source: Elaborated by authors.

Activity on development, survival, and fertility

Eggs from females maintained on plants treated with azadirachtin (3.9 days) showed a significantly lower incubation period than observed in the control (4.1 days). The larval phase lasted 2.1 days in plants treated with azadirachtin and 1.4

in control plants. The duration of the protonymph and deutonymphs phases were not affected by azadirachtin. The adult viability was lower in the treatment with azadirachtin (50%) than observed in the control treatment (63.3%). The mean egg-adult duration was significatively higher for mites reared on plants treated with azadirachtin (10.7 days) than for mites from control plants (9.9 days) (Table 3). It is possible that the rapid absorption of the azadirachtin by the roots can explain the effect on the *O. yothersi* larvae and nymph viability (SUNDARAM et al., 1995).

Table 3. Mean duration (in days ± standard error) and viability (%) of immature stages of *Oligonychus yothersi* feeding on yerba mate leaves treated with 30 mg a.i.·L^{-1} of azadirachtin via systemic irrigation in the laboratory (26 ± 1 °C, RH = 60 ± 10%, and 12 h of photophase)#.

Treatment	Mean duration (days)					
	N*	Egg	Larva	Protonymph	Deutonymph	Duration Egg-adult
Azadirachtin 30 mg a.i.·L^{-1}	30	3.90 ± 0.06 B	2.10 ± 0.22 A	1.90 ± 0.16 A	2.20 ± 0.17 A	10.70 ± 0.21 A
Viability (%)		100	83.30	84.00	71.40	50.00
Control	30	4.10 ± 0.08 A	1.40 ± 0.12 B	1.60 ± 0.10 A	1.80 ± 0.15 A	9.90 ± 0.19 B
Viability (%)		100	96.70	86.20	76.00	63.30

N: number of mites evaluated; #mean values (± standard error) followed by the same letter in the column do not statistically differ from each other according to Tukey's test ($p < 0.05$); RH: relative humidity. Source: Silva (2020).

Fertility, pre-oviposition phase, and male longevity were negatively affected by azadirachtin (Table 4), as stated by MORDUE; NISBET (2000).

Table 4. Fertility (total number of eggs/female ± standard error) and duration in days of the pre-oviposition and oviposition periods, and longevity of *Oligonychus yothersi* maintained on yerba mate leaves treated with 30 mg·L^{-1} of azadirachtin and distilled water (control) via systemic irrigation in the laboratory (26 ± 1 °C, RH = 60 ± 10%, and 12 h of photophase)#.

Parameter	Treatment			
	N	Control	N	Azadirachtin
Fertility	11	34.00 ± 6.12 A	10	19.40 ± 2.95 B
Pre-oviposition	14	2.40 ± 0.17 A	10	1.90 ± 0.10 B
Oviposition	11	9.50 ± 1.60 A	10	8.10 ± 1.45 A
Female longevity	14	10.00 ± 1.66 A	10	12.00 ± 1.42 A
Male longevity	5	18.00 ± 1.30 A	5	9.60 ± 3.08 B

N: number of mites evaluated; #mean values (± standard error) followed by the same letter in the column do not statistically differ from each other according to Tukey's test ($p < 0.05$); RH: relative humidity. Source: Silva (2020).

The systemic activity of azadirachtin was previously proved in white spruce [*Picea glauca* (Moench) Voss] seedlings after immersing their roots in a nutrient solution containing azadirachtin-A (SUNDARAM, 1996). Also, azadirachtin solution was injected into the litchi tree (*Litchi chinensis* Sonn.) trunk, and it was detected in the fruits. Thus, uptake, translocation, persistence, and dissipation of azadirachtin by the plant tissues were observed (SCHULTE et al., 2006).

Additionally, azadirachtin solution was applied to the soil, near the potted aspen plants roots (*Populus tremuloides* Michx.). Also, *T. urticae* was controlled in the foliage from aspen plant treated with azadirachtin. Thus, the compound was taken up from the root system to stem and foliage in 3 h, confirming that azadirachtin is systemic (SUNDARAM et al., 1995). Azadirachtin also applied in the soil is absorbed systemically by bean and coconut plant roots, thus affecting mites that feed on the leaves (SUJATHA et al., 2005; THOEMING et al., 2006), as observed in the present study.

Because azadirachtin is sensitive to photodegradation, applying it via soil irrigation represents an advantage against spraying, as there is lower interference of environmental factors, thus enabling longer protection for the treated plants and lower impact to non-target organisms, and the environment (WEINTRAUB; HOROWITZ, 1997; SOUZA; VENDRAMIM, 2005).

As observed in this study, ALVES et al. (2016) previously found the direct and residual effect of spraying azadirachtin 30 mg a.i.·L^{-1} on yerba mate leaf discs against adults of *O. yothersi*, reducing the mite survival and egg hatching. Besides, in-field test the mite population had decreased by 59.6% at 14 days after the first application of azadirachtin.

Aside from acaricidal activity, the product was safe for the yerba mate plant, as no phytotoxicity was observed in the plants, thus confirming the product's potential as a sustainable alternative for managing populations of *O. yothersi*. For effective use of azadirachtin on yerba mate plants, it is important that further tests are conducted to evaluate potential effects on natural enemies and other organisms associated with the crop, to determine the safety margin for applications. Further studies in field condition, using developed plants irrigated with the recommended dose of azadirachtin, could support the results of the present study. It would also be interesting to analyze product persistence to recommend the use of this product for the control of *O. yothersi*.

CONCLUSIONS

Biological parameters both of red mite male and female were affected by feeding on Azamax-irrigated yerba mate plants.

AUTHORS' CONTRIBUTIONS

Conceptualization: Alves, L.F.A.; Rode, P.A. **Data curation:** Rode, P.A. **Formal analysis:** Rode, P.A. **Funding acquisition:** Alves, L.F.A. **Investigation:** Alves, L.F.A.; Rode, P.A. **Methodology:** Rode, P.A.; Loeblein, J.S. **Project administration:** Alves, L.F.A. **Resources:** Alves, L.F.A. **Supervision:** Alves, L.F.A. **Validation:** Alves, L.F.A.; Toldi, M. **Visualization:** Alves, L.F.A.; Rode, P.A.; Toldi, M. **Writing – original draft:** Rode, P.A.; Toldi, M. **Writing:** Alves, L.F.A.; Rode, P.A.; Toldi, M.; Ferla, N.J.

AVAILABILITY OF DATA AND MATERIAL

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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CONFLICTS OF INTEREST

The authors declare no conflict of interests.

ETHICAL APPROVAL

Not applicable.

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