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Azadirachtin on *Oligonychus yothersi* in yerba mate *Ilex paraguariensis*

Efeito da azadiractina sobre *Oligonychus yothersi* (McGregor) (*Acari*: Tetranychidae) na cultura da erva-mate (*Ilex paraguariensis* St Hil.)

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ABSTRACT

*The red mite **Oligonychus yothersi** is one of the main pests of yerba mate in Brazil The damage this mite causes leads to leaf drop and decreased production. There are no registered acaricides for use in yerba mate; thus, laboratory and field experiments were performed to evaluate the effect of azadirachtin (Azamax®, 250mL 100L^{-1}) for the control of the red mite in yerba mate. In the laboratory, azadirachtin was applied to yerba mate leaf disks before (residual contact) and after (direct contact) infestation with 15 newly emerged red mite adult females. The effect of azadirachtin on mite behavior was evaluated in arenas with treated and untreated yerba mate leaves, and the number of mites in both areas was recorded. Ovicidal action was evaluated by applying azadirachtin to eggs and recording egg hatching. In the field, two applications of the product were performed (1L spray liquid plant^{-1}) with a 7-day interval. The numbers of living mites were evaluated at 7, 14 and 21 days following the first application on randomly collected leaves. It was observed 86.6 and 91.4% of mortality following 24h of residual and direct contact, respectively. Repellent (62% of individuals leaving the treated area) and ovicidal (98.9% decrease in egg hatching) effects were also observed. The mite population in the yerba mate crop field had decreased by 59.6% at 14 days after the first application of azadirachtin. The results show the potential of azadirachtin for the control of **O. yothersi** in yerba mate in Brazil.*

Key words: *neem, alternative control, yerba mate pest.*

RESUMO

*O ácaro **Oligonychus yothersi** é uma das principais pragas da cultura da erva-mate. Seus danos levam à queda das folhas e redução da produção. Não há acaricidas registrados para uso na erva-mate e por isso foram realizados experimentos em laboratório e a campo para avaliar o efeito da azadiractina (Azamax®, 250mL 100L^{-1}), visando ao controle da espécie na cultura da erva mate. Em laboratório, o produto foi aplicado em discos de folha de erva-mate antes (contato residual) e depois (contato direto) da infestação com 15 fêmeas adultas recém-emergidas do ácaro vermelho. A ação do produto no comportamento do ácaro foi avaliada em arenas com folhas de erva-mate tratadas e não tratadas, registrando-se o número de ácaros em ambas as áreas. Além disso, a ação ovicida foi avaliada, aplicando-se o produto sobre ovos e registrando a sua eclosão. No campo, foram realizadas duas aplicações do produto (1L de calda planta^{-1}), espaçadas sete dias. Avaliou-se o número de ácaros vivos, aos 7, 14 e 21 dias após a primeira aplicação, em folhas coletadas aleatoriamente. Observou-se ação acaricida (86,6 e 91,4% de mortalidade após 24h, respectivamente, por contato residual e direto; repelência (62% dos indivíduos abandonando a área tratada) e ação ovicida (redução de 98,9% na eclosão). Na lavoura de erva-mate, a redução populacional do ácaro foi de 59.6% aos 14 dias após a primeira aplicação do produto. Os resultados demonstram o potencial de emprego da azadiractina para o controle de **O. yothersi** na cultura da erva-mate.*

Palavra-chave: *nim, controle alternativo, praga da erva-mate.*

INTRODUCTION

Oligonychus yothersi (avocado red mite) is a polyphagous mite occurring in avocado, coffee, eucalyptus, mango, ornamental plants and yerba mate (MORAES & FLECHTMANN, 2008). In yerba mate, this species occurs under conditions of high-density cultivation. Its colonies are found on the adaxial side of older leaves (mature) (SILVA et al., 2001), causing crinkling and dark spots due to leaf necrosis and leading to early leaf drop

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(ALVES et al., 2004). In Brazil, no miticides are registered for the control of this mite in yerba mate (AGROFIT, 2015), which encourages the use of chemical insecticides by growers. However, there is restriction concerning the presence of toxic residues in leaves used in infusions. Alternatives are therefore needed, such as insecticidal plants (PASINI et al., 2003) and biological control with entomopathogenic fungi (OLIVEIRA et al., 2004).

Neem (***Azadiracta indica***) is a plant of Asian origin with well-known insecticidal action causing feeding inhibition, repellency, interference with pest growth and a decrease in fertility and fecundity (MORDUE (LUNTZ) & NISBET, 2000). Neem insecticides are safer than chemical for human health and the environment being less toxic to predatory mites in different crops (SCHLESENER et al., 2013; BERNARDI et al., 2013). However, the composition of neem-based commercial products in Brazil varies according to the origin of plants used in the formulation. For this reason, a standardized product with a high degree of purity is desirable (BRITO et al., 2006; BERNARDI et al., 2013).

Azadirachtin (Azamax® 12g L^{-1}) was the first insecticide registered for pest control in Brazil being also authorized for use in organic agriculture (IBD, 2015; AGROFIT, 2015). However, there is no information on its effects to control ***O. yothersi*** in yerba mate. In this study, the effect of azadirachtin on ***O. yothersi*** was evaluated in the laboratory and in a commercial cultivation of yerba mate.

MATERIALS AND METHODS

Oligonychus yothersi rearing*:* laboratory population of ***O. yothersi*** was established in yerba mate seedlings grown in pots containing soil + humus and maintained in a climate-controlled greenhouse (27±1°C, 60-70% relative humidity).

The commercial product Azamax® (azadirachtin A/B 12g L^{-1}, inert ingredients 988g L^{-1}; UPL Brazil) was used in all experiments at a concentration of 250mL of commercial product per 100L of water.

Mortality of red mite adults: yerba mate leaves were immersed in 1% sodium hypochlorite for 1 minute and then washed with distilled water. Leaf disks of 226 mm^2 were obtained using a metal hole puncher. For the evaluation of the effects of direct contact, 15 newly emerged and unsexed adult mites were placed on the adaxial side of disks, using a thin soft brush made of hair (size 00). Disks were placed in Petri dishes and sprayed with 1mL of 0.25% commercial product, using a Potter spray tower (0.7 kgf cm^{-2}) (approximately 0.02mL cm^{-2}). Control disks were sprayed with distilled water. Following spraying, the disks were transferred into Petri dishes containing a layer of polyacrylamide gel and placed in a growth chamber (25±1ºC, 60±10% relative humidity and 12h light:12h dark photoperiod). Twelve leaf disks were used (replicates) for azadirachtin and control treatment. For evaluation of the effects of residual contact, the same procedure was adopted, but the mites were placed on the leaf disks after the liquid had been sprayed and allowed to dry.

Mortality was evaluated 24 h after the azadirachtin application using a stereomicroscope. Mites that did not respond to the touch of the brush were considered dead.

Effect on eggs*:* a total of 24 yerba mate leaf disks were used. Twenty adult females of ***O. yothersi*** were placed on each leaf disk for 24h to allow egg laying. Mites were then removed, and 15 eggs were left on each disk, with the excess of eggs being removed by punching. Twelve disks were then sprayed with commercial product 0.25%, and the remaining were sprayed with distilled water (control). Following drying (approximately 30 minutes), the disks were transferred into Petri dishes containing polyacrylamide gel and incubated. The number of hatched larvae was evaluated daily for 7 days using a stereomicroscope.

Repellent effect: following cleaning and disinfection, yerba mate leaves were cut into rectangles (2×4cm, width × length) forming an arena divided longitudinally by the central nerve. One-half of the leaf was immersed in azadirachtin for 15 seconds, and the other was left untreated. The leaves were placed horizontally and dried in the shade. Ten unsexed adult mites were then placed on the central nerve of the adaxial side of each leaf. Twelve replicates (leaves) were used. Evaluations were performed at 5 min and at 1, 6, 12 and 24 h following mite release, using a stereomicroscope. Numbers of mites present in the treated and untreated areas were recorded.

Evaluation in yerba mate field cultivation: the experiment was conducted in a commercial cultivation of yerba mate on an organic farm located in Chopinzinho, state of Paraná (25°51'54.63"S, 52°35'05.38"W, 809m). Twenty trees of similar size and height (1.5 to 2m) and 15 years of age were randomly selected, with 2m distance between the plants and 3m between the rows. Ten plants were used for the azadirachtin and control treatments.

Population sampling was performed in the selected plants on August 28, 2013 before

application of the azadirachtin, with collection of 20 mature leaves infested with ***O. yothersi*** (ALVES et al., 2004). Mite presence in the leaves was evaluated at the laboratory with a stereomicroscope. The first application of azadirachtin was performed using 1L of spray liquid/plant using a sprayer with a D6 nozzle and coupled to a tractor (21 kgf cm^{-2}). A second azadirachtin application was performed after 7 days. The leaf mite populations were evaluated at 9, 14 and 21 days following the first application.

Daily temperatures (minimum, medium and maximum) and rainfall for the experimental period were obtained from the Simepar <www.simepar.br>.

Statistical analysis: a completely randomized experimental design was used. The laboratory experiments were repeated three times for confirmation of the results. For the field experiment, a split-plot in time experimental design was used, with the split-plots corresponding to the different times following treatment application.

The mortality from direct or residual action data was compared with Student's t-test ($P>0.05$), using Microsoft Excel® software. Data obtained from all experiments were tested for normality (Shapiro-Wilk), found to meet the assumptions and subjected to an analysis of variance, followed by Tukey's test ($P<0.05$), using Sisvar software (FERREIRA, 2011).

The Repellency Index (RI) proposed by Lin et al. (1990) was estimated to compare the response among treatments and calculated with the equation RI = 2G/(G + P) where G = is the % mites in the treated area and P is the % mites in the control area. Values were classified as RI = 1 neutral plant, RI < 1 repellent plant, and RI > 1 attractive plant. As a safety margin for that classification, the value of the standard error for each treatment was added/subtracted.

The efficiency of azadirachtin in the laboratory was calculated using the Schneider-Orelli formula and in the field using the Henderson-Tilton formula <http://www.ehabsoft.com/ldpline/onlinecontrol.htm>.

RESULTS AND DISCUSSION

Mortality of red mite adults: Azadirachtin presented direct and residual miticide action. In the direct-effect assay, mites presented decreased movement immediately following azadirachtin application, and the mite population decreased by 91.6% after 24h. In the residual-effect assay, mites started moving more intensely following placement in leaves treated with azadirachtin resulting in 88.4% mortality after 24h. These values were lower than those observed for the direct effect (91.6% mortality) (Table 1). The mortality observed for the control treatment may have been due to mite handling because no significant differences between the two assays were observed, varying between 7.7 and 8.3%.

Effects of azadirachtin on ***O. yothersi*** have been previously observed. Application of a commercial neem oil formulation (1500 ppm azadirachtin) at 1% to second instar nymphs was observed to cause 77% nymph mortality and decreased fecundity in the surviving adults (PASINI et al., 2003). In the present study; however, a higher mortality percentage was obtained using a commercial product in a concentration 75% lower. This can be attributed to the standardized formulation of the commercial product (BRITO et al., 2006; BERNARDI et al., 2012).

Similar effects of azadirachtin have been reported for the two-spotted spider mite ***Tetranychus urticae*** (Koch) (Acari: ***Tetranychidae***), but in that study, azadirachtin was used at higher concentrations (0.3 to 3.6%) (BERNARDI et al., 2012; SCHLESENER et al., 2013).

Effects on eggs*:* the percentage of nymphs produced was 1.1% with azadirachtin treatment

Table 1 - Mortality of adult red mites, ***Oligonychus yothersi***, in yerba mate 24h after the application of azadirachtin (0.25%) in the laboratory (25±1ºC and 12h light/12h dark photoperiod).

Treatment	Direct contact	Efficiency[1] (%)	Residual contact	Efficiency[1] (%)	Egg hatching (%)
Azamax®	91.6 ± 3.64 a[*]	90.8	88.4 ± 11.63 a	87.4	1.1 ± 3.75b
Control	8.3 ± 4.94 b	-	7.7 ± 6.24 b	-	93.8 ± 4.39[a]
CV (%)	26.67		13.51		8.77

Averages (± SE) followed by the same letter within the same column are not significantly different according to the Tukey test ($P<0.05$); CV = coefficient of variation.

[1]Efficiency calculated using the Schneider-Orelli formula.

[*]statistically significant difference between direct and residual effects according to Student's t-test ($P<0.05$).

and 93.8% for the control treatment (Table 1). Azadirachtin can cause partial or complete egg sterility (SCHMUTTERER, 1990), and no hatching of ***T. urticae*** eggs treated with Neemseto® has been observed, independently of the concentration tested (0.25, 0.5 and 1%) (BRITO et al., 2006). In addition, azadirachtin sprayed on ***T. urticae*** eggs decreased hatching by 50 to 70% (SCHLESENER et al., 2013). This is the first information related to azadirachtin on ***O. yothersi*** eggs. It is likely that azadirachtin pass through the chorion membrane, affecting both cell division and protein synthesis, making embryos inviable (MORDUE (LANTZ) & NISBET, 2000).

Repellent effect*:* according to the repellency index, azadirachtin went from neutral to repellent 1h after the release of the mites and maintained its repellency during the experiment. However, the percentage of repelled mites was not significantly different from the control until 12h after mite release. After 24h, the percentage of repelled mites was approximately twice the initial value (increasing from 48 to 90%) and was significantly different from the control. Furthermore, many mites were observed in the water surrounding the disk (Table 2).

BRITO et al. (2006) also observed the repellent effects of azadirachtin (Neemseto®, 0.25, 0.5 and 1%) against ***T. urticae***, with 96 to 98% repellency. However, these values were observed 3 days after mites were placed on the azadirachtin-treated areas. Regarding the observed mite escape, neem-based products possess translaminar properties (SOUZA & VENDRAMIN, 2005), and the product may have spread from the treated to the untreated half of the leaf a few hours after application, making the whole disk inadequate and forcing the mites to escape into the surrounding water. This was not observed by BRITO el al. (2006), using semicircles leafs, immersed and not immersed in the treatment solution, with subsequent mite release.

Evaluation in yerba mate field cultivation*:* the average number of mites was not significantly different between the plants used for the Azamax® and for the control treatment (average 15.9 and 13.4 mites /leaf^{-1}, respectively; table 3). However, it was observed a significantly decreased in the mite population 7 days after the first application (46.4% efficiency). No significant changes were observed in the mite population in the untreated plants (9.9 mites leaf^{-1}). Fourteen days after the first application, the efficiency of the treatment reached 59.6% and the population in the untreated plants practically doubled, with an average of 20 individuals per leaf (Table 3).

In the last evaluation, 21 days after the first application, the mite population in the Azamax® treated and control plants was lower than the initial populations, with no difference existing between treatments. During September 15 and 23 in 2013 (between the second and third population evaluation) it was observed a higher rainfall when compared with the previous weeks (Figure 1). It is possible that both the product and the mites were removed by the rain explaining the results observed. Negative effects of rain on mite populations have been previously observed for ***O. ilicis*** on coffee plants (FRANCO et al., 2008; PEDRO NETO et al., 2010), and for ***O. yothersi*** (ALVES et al., 2004).

When applied at a concentration of 1.2%, azadirachtin has been observed to be effective for the control of ***T. urticae*** in strawberry crops, with effectiveness levels similar to the ones observed in the present study (BERNARDI et al., 2012). However, the action of azadirachtin may be considered slow considering the growth potential of mite populations because the surviving mites

Table 2 - Repellent effect of azadirachtin (0.25%) on ***Oligonychus yothersi*** on yerba mate leaves (25±1ºC and 12h light/12h dark photoperiod).

Time following mite release (h)	Repelled mites (%)	Repellency index	Classification
0.25	48 ± 8.0 b	1	Neutral
1	62 ± 8.0 b	0.8	Repellent
6	72 ± 3.7 ab	0.9	Repellent
12	72 ± 8.6 ab	0.7	Repellent
24	90 ± 4.4 a	0.5	Repellent
CV (%) = 24.48			

Averages (± SE) followed by the same lower case letter within the same column are not significantly different according to Tukey's test (P<0.05); CV = coefficient of variation.

Table 3 - Average number of living red mites, ***Oligonychus yothersi***, following the application of azadirachtin 0.25% to yerba mate leaves under field conditions (Chopinzinho, Paraná, August - September 2013).

Plants	Days following the first Azamax application						
	Pre-sampling*	7**		14		21	
	n	n	Efficiency (%)	n	% C	n	% C
Control	13.4 ± 1.33 abA	9.9 ± 1.37 bcA	-	20.0 ± 3.4 aA	-	2.6 ± 0.4 cA	-
Treated	15.9 ± 2.94 aA	7.5 ± 1.74 cA	33.5	9.6 ± 1.9 abB	59.6	2.9 ± 0.5 cA	21.6
CV 1 (%) = 31.5							
CV 2 (%) = 21.39							

Averages (± SE) followed by the same lower case letter within the same line and upper case within the same column are not significantly different according to the Tukey test, at $P<0.05$.

*First application of Azamax®.

**Second application of Azamax®.

% C = Efficiency calculated using the Henderson-Tilton formula.

C.V. 1 = coefficient of variation for comparison between different times; C.V. 2 = coefficient of variation for comparison between the Azamax and control treatments.

can produce progeny in 7 days (ALVES et al., 2004). Therefore, reapplication may be necessary to increase the azadirachtin effects on mites due to the addition of new individuals in the population (BERNARDI et al., 2012).

The observed decrease in ***O. yothersi*** populations 7 days after azadirachtin application is in accordance with the occurrence of a translocation peak on the plant for up to 5 days and storage in leaves for up to 8 days (MARTINEZ, 2002). In addition, the action of neem products decreases with time, which is attributed to environmental factors mainly temperature, sunlight UV radiation and rain (CABONI et al., 2006; BERNARDI et al., 2012; SCHLESENER et al., 2012). The short residual action of the product should be compensated with reapplication at 7 days intervals (CABONI et al., 2006; BERNARDI et al., 2012), which was also observed for the yerba mate crop.

In the case of yerba mate, the absence of toxic residues after application of Azamax® is an advantage because the leaves are used for beverages and extraction of caffeine and others derivatives. In addition, the use of azadirachtin has a reduced effect on predatory mites being an alternative for conservative biological control (BRITO et al., 2006; BERNARDI et al., 2012).

CONCLUSION

Azadirachtin 0.25% has effect on ***O. yothersi*** eggs and adults, causing mortality and repellency being an alternative to control ***O. yothersi*** in yerba mate crops.

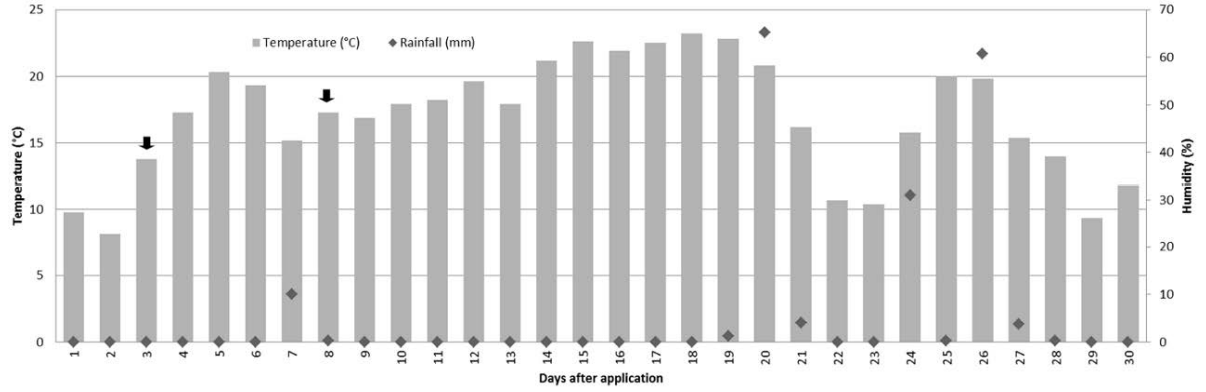

Figure 1 - Temperature (°C) and rainfall (mm) during field experiment (Chopinzinho, PR (August/26/ 2013 – September/26/2013, from: SIMEPAR, 2013) (= ⬇ product application).

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