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EFFECT OF AQUEOUS EXTRACT OF *Prosopis juliflora* ON THE CONTROL OF THE MITE *Tetranychus bastosi* IN PHYSIC NUT[1]

MARIA DA PENHA MARTINS DO NASCIMENTO[2]*, CARLOS ROMERO FERREIRA DE OLIVEIRA[2], CLÁUDIA HELENA CYSNEIROS MATOS[2], CÉSAR AUGUSTE BADJI[3]

ABSTRACT - The objective of this study was to evaluate the efficiency, phytotoxicity and residual effect of the aqueous extract of leaves of *Prosopis juliflora* (Sw.) DC (Fabaceae) for *Tetranychus bastosi* Tuttle, Baker & Sales (Acari: Tetranychidae) on *Jatropha curcas* L. (Euphorbiaceae). For the evaluation of the control efficiency, physic nut plants were infested with 30 adult mite females and after 12 days were sprayed with the lethal concentrations (m/v) of the extract (LC_{50} = 53.45% or CL_{90} = 85.35%) and with distilled water (control), which corresponded to the treatments. After 24, 48, 72, 96 and 120 hours after application of the extract, two leaves of the lower, middle and upper third of the plants were sampled and the live mites were counted per treatment. The evaluation of the residual effect was done three, 24, 48, 96, 192 and 288 hours after spraying. To evaluate the phytotoxic effect of the extract, scores were given according to the intensity of the symptoms in the plants. Control efficiency was verified throughout the evaluated period, with an average of 81.67% for LC_{50} and 73.05% for LC_{90}, with no significant difference between the evaluation intervals. The extract had a low residual effect on *T. bastosi*, but at the end of 12 days the average percentage of oviposition reduction of mite was 49.21% and 68.86% for LC_{50} and LC_{90}, respectively. The plants did not present phytotoxicity. *P. juliflora* extract presents potential for the alternative control of *T. bastosi* in physic nut due to its efficiency on the mortality of this mite, oviposition reduction of females and absence of phytotoxic effect in the plants.

Keywords: Natural acaricide. Residual effect. Tetranychidae. Mesquite. Phytotoxicity. *Jatropha curcas*.

EFEITO DO EXTRATO AQUOSO DE *Prosopis juliflora* NO CONTROLE DO ÁCARO *Tetranychus bastosi* EM PINHÃO-MANSO

RESUMO - O objetivo deste trabalho foi avaliar a eficiência, fitotoxicidade e efeito residual do extrato aquoso de folhas de *Prosopis juliflora* (Sw.) DC. (Fabaceae) para *Tetranychus bastosi* Tuttle, Baker & Sales (Acari: Tetranychidae) em *Jatropha curcas* L. (Euphorbiaceae). Para a avaliação da eficiência de controle plantas de pinhão-manso foram infestadas com 30 fêmeas adultas do ácaro e após 12 dias, foram pulverizadas com as concentrações letais (m/v) do extrato (CL_{50} = 53,45% ou CL_{90} = 85,35%) e com água destilada (testemunha), correspondendo aos tratamentos. Decorridas 24, 48, 72 e 96 e 120 horas após a aplicação do extrato, duas folhas do terço inferior, médio e superior das plantas foram amostradas e contabilizou-se os ácaros vivos por tratamento. A avaliação do efeito residual foi feita três, 24, 48, 96, 192 e 288 horas após a pulverização. Para avaliação do efeito fitotóxico do extrato foram atribuídas notas de acordo com a intensidade dos sintomas nas plantas. Constatou-se eficiência de controle por todo período avaliado, com média de 81,67% para a CL_{50} e 73,05% para CL_{90}, sem diferença significativa entre os intervalos de avaliação. O extrato apresentou baixo efeito residual sobre *T. bastosi*, mas ao final de 12 dias a porcentagem média de redução da oviposição do ácaro foi de 49,21% e 68,86% para a CL_{50} e CL_{90}, respectivamente. As plantas não apresentaram fitotoxicidade. O extrato de algarobeira apresenta potencial para o controle alternativo de *T. bastosi* em pinhão-manso em razão de sua eficiência sobre a mortalidade deste ácaro, redução da oviposição de fêmeas e ausência de efeito fitotóxico nas plantas.

Palavras-chave: Acaricida natural. Efeito residual. Tetranychidae. Algaroba. Fitotoxicidade. *Jatropha curcas*.

*Corresponding author
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[2]Academic Unit of Serra Talhada, Universidade Federal Rural de Pernambuco, Serra Talhada, PE, Brazil; mariapenhan@yahoo.com.br - ORCID: 0000-0001-7074-3238, carlos.foliveira@ufrpe.br - ORCID: 0000-0001-8250-6344, claudia.matos@ufrpe.br - ORCID: 0000-0001-5040-2479.
[3]Academic Unit of Garanhuns, Universidade Federal Rural de Pernambuco, Garanhuns, PE, Brazil; cesar.badji@ufrpe.br - ORCID: 0000-0001-8082-3784.

INTRODUCTION

The mite *Tetranychus bastosi* Tuttle, Baker & Sales (Acari: Tetranychidae) is considered the main pest mite of the jatropha in Brazil (SARMENTO et al., 2011; CRUZ et al., 2012). The control of this mite is made with synthetic acaricides without registration for culture in the Ministry of Agriculture, Livestock and Supply, which is worrisome due to the possibility of adverse effects resulting from its use, such as population reduction of natural enemies and the development of resistant mite populations (ESCUDERO; FERRAGUT, 2005; VENZON et al., 2010). In this sense, there is a growing demand for sustainable control methods, with technologies that have a lower impact on the environment (OLIVEIRA et al., 2007), and the use of plant extracts for the control of pest arthropods (MOREIRA et al., 2005).

The use of substances of vegetal origin in pest management has established, including well-known products such as neem (MOREIRA et al., 2005; VENZON et al., 2010; SILVA et al., 2010). In addition, the use of plant extracts in pest management is an alternative to the use of synthetic acaricides because of their repellent properties, short residual effect and low toxicity to humans (CHIASSON; BOSTANIAN; VINCENT, 2004). It is also worth mentioning that, depending on the species of plant selected for use, the extract can be produced by the farmers themselves to be applied in their cultivated areas (DEQUECH et al., 2008).

In the semi-arid region, the potential of plant extracts of species commonly occurring in the region was evaluated in the studies of Siqueira et al. (2014), Xavier et al. (2015) and Ferraz et al. (2017) for the control of phytophagous mites.

In this sense, the present study had the objective of evaluating the efficiency, phytotoxicity and residual effect of the aqueous extract of leaves of *Prosopis juliflora* (Sw.) DC. (Fabaceae) in the control of the mite *T. bastosi* in physic nut. The *P. juliflora* is widely distributed in the semiarid region and was introduced in Brazil for fodder and wood crops. It is considered an invasive species, which can be explained, at least in part, by its allelopathic activity from leaf tissues *in vivo* or in soil decomposition. Studies have demonstrated the bactericidal, nematicidal, fungicidal and cytotoxic potential of this type of extract (BATATINHA et al., 2011; SHEIKE et al., 2012; VEDAK; RAUT, 2014), which reinforces the importance of research that assesses its potential on other organisms, such as phytophagous mites.

MATERIAL AND METHODS

The experiments were conducted at the Laboratory of the Nucleus of Arthropod Ecology of the Academic Unit of Serra Talhada (NEA/UAST/ UFRPE), Universidade Federal Rural de Pernambuco (UFRPE).

Mite Cultivation - *T. bastosi* mites were cultivated on Gerbox®-type plaques containing arenas of leaves of *Canavalia ensiformes* (L.) DC (Fabaceae) placed with the adaxial side facing downwards on a layer of polyethylene foam (4 cm thick), constantly moistened with distilled water. The water, besides being a barrier to the escape of the mites, kept the turgescence of the leaf (REIS; ALVES; SOUSA, 1997). Hydrophilic cotton was used to cover the entire edge of the leaves, avoiding the escape of the mites to the adaxial side of the leaf. When necessary, the leaves were replaced with others and the mites transferred with the aid of a brush. The cultures were kept in climatic BOD Incubator (27 ± 2 ° C, 70 ± 5% RH and 12-hour photophase).

Preparation of the extract - The leaves used to extract the *P. juliflora* extract were from trees located in the vicinity of UAST/UFRPE. The samples were collected in the morning and the plants were in the flowering and fruiting phase. All the collected material was packed in paper bags, properly labelled, and taken to the NEA/UAST for later use in the bioassays. Voucher material is stored in the Brazilian Semiarid Herbarium (HESBRA) (Voucher 3512).

After harvesting, the plant material was disinfected in 0.05% active chlorine solution for 20 minutes (VIEIRA et al., 2006). It was then washed in distilled water and dried in an oven (45°C) for 48 hours. Subsequently, this material was ground with the aid of a crusher, and the powder obtained was weighed to be used to obtain the aqueous extract.

The concentrations of the extracts (m/v) used in the preliminary bioassays were 0%, 5%, 15%, 25%, 35%, 45% and 55%, obtained based on the dehydrated leaf weight ratio for each 100 mL of distilled water (0, 5, 15, 25, 35, 45 and 55g of leaf/100ml). The material was conditioned in the refrigerator (at a medium temperature of 5°C) for a period of 24 hours, until the raw extract was obtained.

In order to determine the lethal concentrations (LC's) of the *P. juliflora* extract that kill 50% (LC_{50}) and 90% (LC_{90}) of the *T. bastosi* population, the average mortality data of the mites at the above mentioned concentrations were submitted to PROBIT analysis, with LC_{50} = 53.45% (m/v) and LC_{90} = 85.35% (m/v), respectively.

Obtaining physic nut plants - The plants were grown in polyethylene pots (5.0 kg capacity), in the ratio of 3:1:1 soil, commercial substrate (Vermiculite) and tanned bovine manure. The seeds used came from the Active Germplasm Bank of the Instituto Agronômico de Pernambuco (BAG - IPA), located in Serra Talhada - PE. Throughout the

experiment the plants were kept in wooden cages (0.5m x 0.5m x 1.60m) coated with voile-like tissue to prevent natural infestation by insects and mites.

Control efficiency of and phytotoxicity of *P. juliflora* aqueous extract - To perform the tests, physic nut plants were infested with 30 adult *T. bastosi* females. After 12 days of infestation, the aqueous extract of *P. juliflora* leaves was sprayed at one of the laboratory determined lethal concentrations: LC_{50} = 53.45% (m/v) or LC_{90} = 85.35% (m/v), in two separate experiments, pointing the jet to the underside of the leaves. The experimental design was completely randomized (for both LC_{50} and LC_{90}), with two treatments: T1 = plant sprayed with *P. juliflora* extract (LC_{50} or LC_{90}); T2 = plant sprayed with distilled water, corresponding to the control and three replicates.

Spraying was done using a commercial costal spray of 5L capacity. In order to verify the application distribution of the *P. juliflora* aqueous extract or distilled water (control) in the plants, hydrosensitive paper cards (26x76mm) were fixed in different positions in the plant. This paper allows to analyse the uniformity of distribution and diameter of the droplets, and to evaluate the quality of application of products in the target (DEBORTOLI et al., 2012).

After 24h, 48h, 72h, 96h and 120 hours of spraying, two (pre-defined) leaves of the lower, middle and upper thirds of the jatropha plants were sampled, totalling six leaves/plant. The counting of the adult females of T. bastosi was done with the aid of an "ocular magnifying glass". The data obtained were used to calculate the agronomic efficiency using the formula of Abbott (1925): Efficiency (E %) = (t - p) / t x 100, where **t** is the infestation in the controls and **p** is the infestation in the plot treated.

To evaluate the plants with symptoms of phytotoxicity, the effects were evaluated 48 hours, 72 hours, 96 hours and 120 hours after the application of the extract, assigning notes according to the intensity of the symptoms, using the adapted scale proposed by Martins et al. (2004). The criterion was established for the percentage of total leaf area reached by the symptoms evaluating the set of leaves of the plant, being: score 0 = no symptoms affected in the leaf area; score 1 = between 1 and 20%; score 2 = between 21 and 40%; score 3 = between 41 and 60%; score 4 = between 61 and 80%; and score 5 = with more than 81% of the leaf area affected.

Residual Efficiency of the aqueous extract of *P. juliflora* on *T. bastosi* - It was done following the same methodology of the test of control efficiency, regarding the application of the extract and infestation of the mites in the plants. However, after the spraying of the extracts in the plants with the LC_{50} or LC_{90}, samples of leaves were collected at intervals of three, 24, 48, 96, 192 and 288 hours after application of the products (ESTEVES FILHO; OLIVEIRA; MATOS, 2013). In the laboratory, leaf disks (3.5 cm Ø) of each treatment were individually arranged in Petri dishes containing foam (1 cm thick) covered by filter paper, with hydrophilic cotton moistened with distilled water placed around to keep moisture. Fifteen adult females of *T. bastosi* were placed on each disc. The arenas were kept in an air-conditioned room at 27 ± 2ºC, 70% RH and 12h of photophase. Mortality was evaluated after 48 hours, and mites that did not move vigorously after a slight touch with a fine brush were considered dead. The experimental design was completely randomized, with two treatments (T1 = leaves sprayed with *P. juliflora* extract, T2 = leaves sprayed with distilled water, corresponding to the control) and five replicates. After 48 h, the number of eggs was also counted, which was used to calculate the percentage of oviposition reduction, using the adapted formula of Obeng-ofori (1995): PR = [NC-NT] / (NC + NT) x 100], PR = average percentage of oviposition reduction, NC = average of eggs in the control and NT = average of eggs in treatment. The corrected mortality was calculated by Abbott's formula (1925): Ma = (Mt-Mc)/(100 - Mc) x 100, where Ma = mortality corrected as a function of the control; Mt = mortality observed in the treatment with extract and Mc = mortality observed in the control. The results were submitted to analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Application of the *P. juliflora* extract to evaluate the control efficiency on the mite *T. bastosi* provided homogeneous cover on the leaves of the physic nut, which was verified by the coloration of the hydrosensitive cards that presented uniform blue coloration in more than 90% of the area, which indicates the coverage of the extract in the plants.

The aqueous extract of *P. juliflora* leaves at LC_{50} = 53.45% was efficient in the control of *T. bastosi* in all evaluated periods, which did not show significant differences between them (Table 1). After 24 hours of the application of the extract, control efficiency was higher than 80% and these values were maintained until the end of the experiment (Table 1). This was not observed for the LC_{90} of the extract, whose efficiency presented values greater than 70% and less than 80% in all evaluated periods (Table 2). These results are relevant because, according to Potenza et al. (2005) mortality values equal to or greater than 80% with use of plant extracts are considered satisfactory.

Table 1. Count and control efficiency (CE) of the aqueous extract of *Prosopis juliflora* leaves (LC_{50}) on the fecundity of adult females of *Tetranychus bastosi* (Acari: Tetranychidae) in *Jatropha curcas*, under nursery conditions, at the Academic Unit of Serra Talhada.

		Count x application time (hours)						
Treatment		0	24	48	72	96	120	Average
Control	0	22.83±7.84	24.33±9.03*	22.83±7.59*	41.50±11.40*	46.00±15.72*	45.17±13.72*	33.78±4.72*
P. juliflora	53.45%	10.83±0.79	4.50±1.82	3.50±1.92	3.50±1.25	6.33±2.23	8.50±2.66	6.19±1.21
(%)	CE		81.50±5.72a	84.62±5.58a	91.56±7.15a	86.22±6.58a	81.18±7.48a	81.67±4.90a

Averages with * in the column differ statistically by ANOVA. Averages followed by the same letter in the row do not differ statistically by the Tukey's Test, at the 5% probability level.

Table 2. Count and control efficiency (CE) of the aqueous extract of *Prosopis juliflora* leaves (LC_{90}) on the fecundity of adult females of *Tetranychus bastosi* (Acari: Tetranychidae) in *Jatropha curcas*, under nursery conditions, at the Academic Unit of Serra Talhada.

		Contagem x tempo de aplicação (horas)						
Treatment		0	24	48	72	96	120	Average
Control	0	35.70±9.01	37.80±7.67*	68.70±17.37*	78.33±16.11*	90.20±22.24*	86.70±18.52*	66.24±9.21*
P. juliflora	85.35%	50.00±9.93	11.30±1.76	8.17±0.60	8.00±1.96	10.30±2.15	19.30±1.54	17.85±6.65
(%)	EC		70.10±7.65a	88.10±10.27a	89.78±20.30a	88.58±12.68a	77.73±17.58a	73.05±13.20a

Averages with * in the column differ statistically by ANOVA. Averages followed by the same letter in the row do not differ statistically by the Tukey's Test, at the 5% probability level.

Another relevant aspect is that, for both lethal concentrations tested, no phytotoxic effect of the *P. juliflora* extract on the physic nut plants was observed. The plants did not present symptoms, obtaining the score 0 (zero), according to the classification of Martins et al. (2004). The same was observed by Ferraz et al. (2017) when using leaf extract of *Ziziphus joazeiro* for the control of the mite *Tetranychus ludeni* in cotton (*Gossypium hirsutum*), whose plants also fit in score 0 regarding the symptoms of phytotoxicity. These results demonstrate a positive characteristic of the aqueous extracts used in these studies, since they have an effect on the target pests without damaging the cultures.

Low persistence of aqueous extract of *P. juliflora* on the mortality of adult females of *T. bastosi* was observed throughout the evaluated period. The corrected mortality rate was close to 6% for both LC_{50} and LC_{90} of the extract, and the average corrected mortality was 2.30 and 2.24, respectively (Tables 3 and 4). This information is important because it allows us to establish in practice at what intervals the extract should be applied for the effective control of the population of the pest.Ferraz et al. (2017), when evaluating the acaricidal activity of the aqueous extract of *Z. joazeiro* on the mortality of adult females of *T. ludeni* in cotton, also observed low values of residual efficiency of the product during the studied period (16 days) whose average corrected mortality was 11.23% - higher than the one found in the present study. This low residual persistence is a characteristic of plant extracts, since they are rapidly degraded by the environment, besides having a broad spectrum of action and being derived from renewable resources (FERRAZ; LOPES; AMORA, 2008). Factors such as light intensity, product exposure time, temperature, humidity, and chemical composition are determinants of its residual efficiency (YU, 2008).

Although *P. juliflora* extract showed a low mortality adult females of *T. bastosi*, it was observed a lower fertility when they were exposed to the extract at the two lethal concentrations tested (Tables 5 and 6). When using the extract LC_{50}, the percentage of fertility reduction varied between the evaluated periods, being higher in the period from one to four days after spraying (Table 5). At the end of the experiment, average percent reduction in fecundity of mite females was 49.21% (Table 5).

As regards LC_{90} of the extract, it was observed that this caused a higher percentage of

oviposition reduction of females of *T. bastosi* than did the LC_{50} (Table 6). In all the evaluated periods this percentage was higher than 50% and, as observed for the LC_{50}, there were periods of one and four days, where the highest percentages of oviposition reduction were observed (Table 6). An explanation for a greater effect of the LC_{90} of the extract on the oviposition of *T. bastosi* may be the fact that when applied at that concentration the extract forms a dense layer on the physic nut leaves reducing the capacity of the mite to feed on the leaf and, consequently, decreases their conversion into progeny. This reduces the population growth of the mite in the crop.

Table 3. Residual Efficiency of LC_{50} of the aqueous extract of *Prosopis juliflora* leaves on the fecundity of adult females of *Tetranychus bastosi* (Acari: Tetranychidae) in *Jatropha curcas*, under nursery conditions, at the Academic Unit of Serra Talhada.

		Mortality (%) x Time of application (hours or days)						
Treatment		0.12d	1d	2d	4d	8d	12d	Average
Control	0	0	0	0	0	0	0.60±0.40	0.1
P. juliflora	53.45%	3.20±0.49*	1.20±0.49*	0.60±0.40	1.00±0.31*	2.00±0.89	6.40±1.86*	2.40*
(%)	Cm	3.2	1.2	0.6	1	2	5.84	2.30

Averages with * in the column differ statistically by ANOVA. [1]Cm = corrected mortality.

Table 4. Residual Efficiency of LC_{90} of the aqueous extract of *Prosopis juliflora* leaves on the fecundity of adult females of *Tetranychus bastosi* (Acari: Tetranychidae) in *Jatropha curcas*, under nursery conditions, at the Academic Unit of Serra Talhada.

		Mortality (%) x Time of application (hours or days)						
Treatment		0.12d	1d	2d	4d	8d	12d	Average
Testemunha	0	0	0	0	0	0.20±0.20	0.40±0.24	0.3
P. juliflora	85.35%	2.00±0.94	1.60±0.67*	1.00±0.77	1.60±0.51*	2.40±0.81*	5.40±0.81*	2.33*
(%)	Cm	2	1.6	1	1.6	2.20	5.02	2.24

Averages with * in the column differ statistically by ANOVA. [1]Cm = corrected mortality.

Table 5. Residual efficiency of aqueous extract of *Prosopis juliflora* leaves (LC_{50}) on the fecundity of adult females of *Tetranychus bastosi* (Acari: Tetranychidae) in *Jatropha curcas*, under nursery conditions, at the Academic Unit of Serra Talhada.

		Fecundity (Quantity) x Application time (hours or days)						
Treatment		0.12d	1d	2d	4d	8d	12d	Average
Control	0	21.40±4.89	44.20±7.21*	48.40±5.70*	52.80±11.69*	50.40±3.36*	50.20±9.06	44.57*
P. juliflora	53.45%	14.20±2.51	6.00±2.55	24.20±6.34	2.80±1.59	15.20±4.27	28.60±8.56	15.17
(%)	PR	20.22	76.10	33.33	89.93	53.66	27.41	49.21

Averages with * in the column differ statistically by ANOVA. [1]PR = average percentage of oviposition reduction.

Table 6. Residual efficiency of aqueous extract of *Prosopis juliflora* leaves (LC_{90}) on the fecundity of adult females of *Tetranychus bastosi* (Acari: Tetranychidae) in *Jatropha curcas*, under nursery conditions, at the Academic Unit of Serra Talhada.

		Fecundity (Quantity) x Application time (hours or days)						
Tratamento		0.12d	1d	2d	4d	8d	12d	Average
Testemunha	0	29.60±3.93*	42.40±5.98*	54.60±8.63*	51.60±7.52*	45.40±8.30*	78.00±10.37*	50.27*
Algarobeira	85.35%	8.00±2.50	3.80±0.97	9.20±3.00	4.20±1.68	8.20±1.28	22.20±3.39	9.27
(%)	PR	57.45	83.55	71.16	84.95	69.40	55.69	68.86

Averages with * in the column differ statistically by ANOVA. [1]PR = average percentage of oviposition reduction.

An important aspect that should be considered in future studies is the viability of eggs of females of *T. bastosi* submitted to these concentrations of the extract, as it is not known in what way the aqueous extract of *P. juliflora* can interfere in new generations of the mite.

Research has demonstrated the effect of plant extracts on the fecundity of adult females of tetraniquids: Lucini et al. (2010) demonstrated that the use of the aqueous extract of *Capsicum baccatum* (Solanaceae) affected the oviposition of adult females of *T. ludeni* (Acari: Tetranychidae), causing a reduction of 25.4% and 34.7%, respectively. The same was observed by Ferraz et al. (2017) using leaf extract of *Z. joazeiro* on this cotton mite, whose percentage of reduction of oviposition of adult females was 10.43%, in a period similar to that carried out in this research.

As far as *P. juliflora* extract is concerned, no research has been done on the use of mite control in the literature. However, studies have shown the nematicidal, fungicidal and bactericidal potential of this type of extract (BATATINHA et al., 2011; SHEIKE et al., 2012; VEDAK; RAUT, 2014). In addition, chromatographic analyses of *P. juliflora* extracts have revealed that their composition varies with the different parts of the plant, and alkaloids, coumarins, steroids, flavonoids, sesquiterpenes, stearic acid, and others may be found, including proteinases inhibiting action (OLIVEIRA et al., 2002; ALMARAZ-ABARCA et al., 2007).

In the specific case of the *P. juliflora* leaves extract used in the present study, the acaricidal activity observed on *T. bastosi* may be due to the presence of phenolic compounds, flavonoids, alkaloids, terpenes and steroids, which are predominant in this part of the plant, as verified by Singh (2012). In fact, these compounds, also found in other plants, are toxic and repellent, acting to inhibit feeding or reproduction, besides reducing growth and survival, or causing death of the various stages of development of arthropods, as verified by several authors (SIQUEIRA et al., 2014; XAVIER et al., 2015; FERRAZ et al., 2017).

CONCLUSION

The aqueous extract of *P. juliflora* leaves showed efficiency in the control of the mite *T. bastosi* in physic nut plants, low residual effect on this mite and did not cause a phytotoxic effect.

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