BioAssay

PLANT EXTRACTS

Effects of the Ethanol Extracts of Leaves and Branches from Four Species of the Genus *Croton* on *Tetranychus urticae* Koch (Acari: Tetranychidae)

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Efeito de Extratos Etanólicos de Folhas e Ramos de Quatro Espécies do Gênero *Croton* Sobre *Tetranychus urticae* Koch (Acari: Tetranychidae)

RESUMO **-** O ácaro rajado *Tetranychus urticae* Koch é uma das principais pragas agrícolas, infestando importantes culturas como o algodoeiro, videira e tomateiro. Nos últimos anos, o estudo de extratos vegetais objetivando o controle alternativo de pragas tem-se intensificado, tornando-se uma alternativa promissora, atraindo o interesse de um número cada vez mais crescente de pesquisadores. Este trabalho teve como objetivo avaliar a efeito residual de extratos etanólicos a 1% de folhas e ramos de quatro espécies do gênero *Croton* (*C. rhamnifolius* Kunth, *C. sellowii* Baill*, C. jacobinensis* Baillon, *C. micans* (Baill)) sobre o ácaro rajado *T. urticae*. Discos de folhas com os ácaros foram previamente imersos por cinco segundos nos extratos. Verificou-se que o extrato das folhas de *C. sellowii* causou 69% de mortalidade, e que o extrato das folhas de *C. jacobinensis* não apresentou toxicidade sobre o ácaro. Os experimentos revelaram ainda que a fecundidade dos ácaros foi afetada e que todos os extratos foram repelentes na concentração de 1%.

PALAVRAS-CHAVE: ácaro rajado, acaricidas vegetais, repelência, *Croton* spp.

ABSTRACT - The two-spotted spider mite *Tetranychus urticae* is one of the principal agricultural pests, infesting important crops such as cotton, grapes and tomatoes. In recent years, studies with the objective of using plant extracts as an alternative pest control are being intensified, showing to be a promising alternative and attracting the interest of a growing number of scientists. The present study aimed to evaluate the residual effect of 1% ethanol extracts of leaves and branches of four species of the genus *Croton* (*C. rhamnifolius* Kunth, *C. sellowii* Baill*, C. jacobinensis* Baillon, *C. micans* (Baill)) on the spider mite *T. urticae*. Leaf disks with the mites were immersed for five seconds in the extracts. It could be verified that the leaf extract of *C. sellowii* caused 69% mortality and the leaf extract of *C. jacobinensis* was not toxic to the spider mite. From the experiments it was concluded that the fecundity of the mites was affected and that the extracts were repellent at a concentration of 1%.

KEY-WORDS: two-spotted spider mite, botanical acaricide, repellence; *Croton* spp.

The uncontrolled use of insecticides in plantations has caused serious damage to the environment (Roel 2001). Therefore, many works are being done to find natural compounds with insecticidal properties (Oliveira *et al.* 1999, Sundaram *et al.* 1995, Pontes *et al.* 2007a, 2007b). In this context the study of the toxic effects of plant extracts on phytophagous mites is interesting (Sundaram *et al.* 1995, Jones *et al.* 1996). The two-spotted spider mite *Tetranychus urticae* Koch is considered one of the most important agricultural pests, infesting crops including cotton, tomatoes and grapes (Moraes & Flechtmann 2008).

As an alternative for synthetic insecticides, plant extracts were heavily investigated in recent years as a source of bioactive substances (Barakat *et al.* 1986a, 1986b, Potenza *et* *al.* 1999a, 1999b, Pontes *et al*. 2007c). Among native species with recognised insecticidal potential, those of the genus *Croton* are outstanding. Species of this genus are common in various biomes of the state of Pernambuco, Brazil. Species of *Croton* are popularly known as "marmeleiro" or "velame" and many are used as drugs for the treatment of hypertension, ulcers and as anti-inflammatories (Maciel *et al*. 2000, Abdel Gadir *et al.* 2003, Nardi *et al.* 2003, Suárez *et al.* 2003, Guerrero *et al.* 2004, Lopes e Lopes *et al.* 2004). The search for insecticidal properties in medicinal plants is growing in the last few years and shows to be a promising alternative (Alexander *et al.* 1991, Roel 2001, Park *et al.* 2002). *Croton cajucara* Benth and *C. linearis* Jacquin have shown medicinal properties (Maciel *et al.* 2000, Almeida *et al.* 2003, Guerrero *et al.* 2004) and from these species secondary compounds with insecticidal properties were isolated (Kubo *et al.* 1991, Alexander *et al.* 1991). According to literature, no works on the acaricidal activity of the species *C. jacobinensis*, *C. sellowii*, *C. rhamnifolius* and *C. micans* have been published.

As part of a systematic study to evaluate the acaricidal potential of flora from the state of Pernambuco, the objective of the present study was to evaluate the activity of ethanolic extracts from branches and leaves of *C. jacobinensis* Baillon, *C. sellowii* Baill, *C. rhamnifolius* Kunth and *C. micans* (Baill) on the spider mite *T. urticae*.

Material and Methods

Biological tests were performed in Laboratório de Biologia de Insetos of the Universidade Federal Rural de Pernambuco (UFRPE) at temperatures of 25 ± 5ºC, RH of 70 ± 8 % and photophase of 12 h. The mites were obtained from the Laboratório de Acarologia of the UFRPE and were maintained on Jack beans (*Canavalia ensiformes* L.) cultivated in greenhouses. The plants were infested after the opening of the first pair of dicotiledonary leaves.

The selected plant species for the study were *C. jacobinensis, C. rhamnifolius C. micans* and *C. sellowii*. Leaves and branches of the first three species were collected from an upland swamp in Brejo da Madre de Deus, Caruaru – PE/Brazil, while the last one was collected in a sandbank at the beach of Gaibú, in Cabo de Santo Agostinho – PE/Brazil. Voucher samples of each collected species were deposited in the Herbarium Vasconcelos Sobrinho of the UFRPE, under the numbers 45553 (*C. jacobinensis*), 45552 (*C. rhamnifolius*), 45209 (*C. micans*) and 45622 (*C. sellowii*).

Extracts were prepared at room temperature in the Laboratório de Produtos Naturais Bioativos of UFRPE. Branches and leaves of the collected species were dried at room temperature, cut, weighted and immersed in a quantity of ethanol enough to cover the plant material for 24 h. This procedure was repeated three times to ensure good extraction of ethanol soluble constituents. The system was filtrated and ethanol was evaporated at reduced pressure in a rotation evaporator to obtain the crude extract.

The crude branch and leaf extracts of the species were dissolved in ethanol to obtain a solution at 1 %. Jack bean leaf disks of 2.5 cm in diameter were immersed for 5 s in the solutions and dried at room temperature. Two controls were used: one in which the leaf disk was immersed in pure ethanol and the other disk immersed in distilled water. The disks were then placed on filter paper upon a polyethylene foam wetted with water and maintained in plastic wares. Each treated disk received 10 adult female spider mites. After infestation, observations were done daily for three days. Mites were considered as dead when they did not move a distance greater than the length of own body after soft contact with a fine haired brush.

To evaluate fecundity, eggs deposited on the disks during 72 h were counted. This experiment consisted of 10 treatments (two controls and two extracts of each of the four species studied). Each treatment was repeated 10 times. The obtained results were submitted to a variance analysis and the mean values were compared by Tukey's test ($p \leq 0.05$) calculated by the program SANEST 3.0.

The repellence tests were performed according to the method described by Kogan & Goeden (1970). Jack bean leaf disks of 4.5 cm in diameter were used. Half of the disk was immersed for 5 s in an ethanol solution of the extracts at a concentration of 1 % and after drying at room temperature, the other half was immersed in pure ethanol serving as control. Each half circle was immersed in such a manner that permitted a free area of 0.3 cm between the two halves, where the mites were initially released. The leaf disks were placed on a filter paper disk, upon polyethylene foam, and the entire arrangement was placed on a plastic tray containing water. Each disk was infested with 10 adult female spider mites and each treatment was replicated 10 times.

Counting the mites present of each half of the leaf was done after 24 h. The mites found in the neutral area during the test were considered repelled or attracted according to their proximity with the control or with the treatment. The repellence index (RI) of the extracts was calculated according to the equation: $RI = 2G/(G + P)$ proposed by Kogan & Goeden (1970), where G = the number of mites in the treated area and P = the number of mites in the control area.

The confidence interval for classifying the extracts was obtained based on the RI mean values and the respective standard deviation (SD). For a mean RI value lower than 1 – SD, the extract is considered as repellent, while for a mean RI value of greater than 1 + SD, the extract is attractive and for the mean value between 1 – SD and 1 + SD the extract is considered as neutral.

Results

Table 1 shows the results for effects of extract mortality as well as those for fecundity of two-spotted spider mites. The extract from *C. sellowii* leaves was one of the most toxic in this series for the spider mites, showing a mean mortality of 69 % in a 72 h exposure interval. The least effective extract was that from *C. sellowii* branches, which resulted in a mean mortality of 6.0 %. The leaf and branch extracts from *C. jacobinensis* showed 9.0 % and 13.0 % mortality,

Table 1. Mortality and fecundity of *T. urticae* treated with ethanolic extracts from branches and leaves of four *Croton* species. Temp. 25 ± 5ºC, RH 70 ± 8 % and photophase 12 h.

Treatment	Plant part	Mean mortality (%)[1]	Mean eggs/leaf disk
Control (ethanol)	-	4.0 ± 2.20 c	304.6 ± 8.29 a
Control (water)	-	5.0 ± 2.20 c	334.7 ± 10.0 a
C. jacobinensis	Leaf	9.0 ± 2.30 c	338.7 ± 9.18 a
	Branch	13.0 ± 4.80 c	275.4 ± 7.84 b
C. rhamnifolius	Leaf	53.0 ± 5.40 ab	160.0 ± 11.60 cd
	Branch	60.0 ± 7.40 ab	118.0 ± 25.0 d
C. sellowii	Leaf	69.0 ± 2.90 a	49.4 ± 12.20 e
	Branch	6.0 ± 3.30 c	176.8 ± 4.80 c
C. micans	Leaf	45.0 ± 6.10 b	169.0 ± 12.80 cd
	Branch	40.0 ± 3.60 b	179.3 ± 7.27 c

[*]Means (± SD) with the same letter in the column do not differ significantly by the Tukey test ($P \leq 0.05$).
[1]Values obtained after 72h of exposure.

respectively, and did not differ significantly from the control ($p \leq 0.05$). The toxicity of leaf extract from *C. jacobinensis* (9.0 %), *C. micans* (45.0 %) and *C. rhamnifolius* (53.0 %) did not differ significantly from those found for branch extracts (13.0 %, 40.0 % and 60 %, respectively).

These results indicated that fecundity was also affected, where the leaf extract from *C. sellowii* showed the best results in this series with the smallest mean number of eggs per leaf disk (49.4, see Table 1). The only extract which did not significantly differ from the control was the extract from *C. jacobinensis* branches. Additionally, there was no difference in the mean number of eggs deposited per mite when submitted to the branch and leaf extracts of *C. rhamnifolius* ($p \leq 0.05$) (118.0 and 160.0/leaf disk, respectively). The same was observed for the *C. micans* leaf and branch extracts (169.0 and 179.3/leaf disk, respectively). All extracts, independent of the tested species, were repellent at a concentration of 1% (Table 2).

Table 2. Mean repellence of ethanol extracts from leaves and branches of four *Croton* species on *T. urticae*. Temp. 25 ± 5ºC, RH 70 ± 8 % and photophase 12 h.

Treatment	Plant part	Repellenc Index[1]	Classification
C. jacobinensis	Leaf	0.27 ± 0.10	Repellent
	Branch	0.48 ± 0.24	Repellent
C. rhamnifolius	Leaf	0.21 ± 0.09	Repellent
	Branch	0.51 ± 0.18	Repellent
C. sellowii	Leaf	0.31 ± 0.11	Repellent
	Branch	0.32 ± 0.99	Repellent
C. micans	Leaf	0.10 ± 0.01	Repellent
	Branch	0.12 ± 0.10	Repellent

[1]Repellence Index calculated according the equation described by Kogan & Goeden (1970)

Discussion

Differences between the toxicity of branch and leaf extracts were also observed by Castiglioni *et al* (2002) for aqueous extracts from seeds and branches of *Azadirachta indica* A. Juss at 5 %. It is quite common that different plant parts show qualitative and quantitative differences in relation to their chemical constituents (Bernays & Chapman 1994). The observed difference between the toxicity of extracts from *C. sellowii* leaves and branches suggests the existence of secondary metabolites present only in leaves or in greater concentrations than in branches. This hypothesis is supported by the results obtained by El-Gengaihi *et al.* (1999), who investigated the acaricidal effect of leaves, seeds and roots of *Glosostemon bruguieri* (Desf). They observed that certain carbohydrates present in leaves are responsible for greater toxicity and that these compounds were not detected in the seeds and roots.

For concentrations of 1 %, neem formulations present toxicity to *Tetranychus cinnabarinus* (Boisd.) (Mansour *et al.* 1986, 1997). Pure azadirachtin is also acaricidal against *T. urticae* (Sundaram & Sloane 1995). Acaricidal activity of aqueous extracts of *Allium cepa* L., *Allium sativum* L., *Stryphnodendron barbatiman* Mart. and *Solanum melogena* L. has already been observed against *T. urticae* (Potenza *et al.* 1999a). Later, the toxicity of extracts of *Annona sp.* (Rodríguez Hernández 2001), *Agave sp.*, *Ruta graveolens* L. and *Dieffenbachia brasiliensis* Veitch against the same mite was also verified (Potenza *et al.* 1999b).

A wide variety of plant species appear to be toxic to *T. urticae* in the extract form (Barakat *et al.* 1986a, 1986b), as liquid fractions (El-Gengaihi *et al.* 1999, 2000) and as isolated compounds, such as colupulone, one of the *Humulus lupulus* L. compounds (Jones *et al.* 1996) and the alkaloid piperoctadecalidine, extracted from *Piper longum* L. (Park *et al.* 2002).

Ethanol extracts from *C. jacobinensis*, despite showing no acaricidal activity and fecundity reduction, are repellent.

These results suggest the existence of bioactive repellent substances which are not necessarily lethal to mites. The plant extracts showing acaricidal performance also showed other activities which affect biological aspects of the mites, such as fecundity, or act on its behaviour, as a repellent. Mansour *et al.* (1986) observed a reduction of 76 % in the fecundity of *T. cinnabarinus*, when submitted to neem extracts. Sundaram & Sloane (1995) also reported a fecundity reduction in *T. urticae* caused by neem extracts, but also reported repellence. Commercial formulations based on neem reduce oviposition of *T. urticae* (Dimetry *et al.* 1993, Mansour *et al.* 1997, Momen *et al.* 1997) and some are also repellents (Momen *et al.* 1997, Mansour *et al.* 1997). Pure azadirachtin reduces the number of eggs laid by *T. urticae* (Martinez-Villar *et al.* 2005). The beta-acids and colupulone compounds were considered as repellents for mites and reduced their survival (Jones *et al.* 1996).

Oviposition reduction may be based on the extract action on mite nutrition (Dimetry *et al.* 1993, Sundaram & Sloane 1995). When incapable of feeding on the leaf, the number of eggs should be reduced as a response to the stress provoked by the extract.

Activity of plant extracts is also affected by the type of solvent used in preparation. Aqueous extracts of neem do not show an effect on *T. cinnabarinus* fecundity while organic extracts are efficient in reduction of fecundity (Mansour *et al.* 1986).

The use of ethanol as an organic solvent for the preparation of *Croton* extracts did not interfere with the test results, as shown in Table 1.

Chagas *et al.* (2003) showed that ethanol, when used as a solvent for crude extract preparation and as a diluent for the test procedure, did not present toxic activity against *Boophilus microplus* (Canestrini), and is versatile to obtain a variety of plant extracts with insecticidal and acaricidal activity. Our results confirm those of Chagas *et al.* (2003) who claimed that ethanol, when used as a solvent, does not interfere with biological activity of plant extracts (Table 1).

These results show that the *Croton* species studied have potential to be used in mite control in the form of ethanolic extracts. The discovery of acaricidal properties in native plant species can aid in future production of safer crops by small farmers, based on application of natural acaricides as a control method against phytophagous mites.

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