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Acaricidal activity of Annonaceae fractions against *Tetranychus tumidus* and *Tetranychus urticae* (Acari: Tetranychidae) and the metabolite profile of *Duguetia lanceolata* (Annonaceae) using GC-MS

Atividade acaricida de frações de anonáceas para *Tetranychus tumidus* e *Tetranychus urticae* (Acari: Tetranychidae) e perfil metabólito de *Duguetia lanceolata* (Annonaceae) por CG-EM

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Abstract

Species of the *Tetranychus* genus feed on plant tissues, which reduces the rate of photosynthesis and can lead to the death of plant tissues. As a result, considerable production losses are caused by these arthropods. Thus, in order to aid in the development of new products for the control of *Tetranychus tumidus* Banks and *Tetranychus urticae* Koch (Acari: Tetranychidae), the initial objective of this study was to select Annonaceae derived fractions that were soluble in dichloromethane and have acaricidal activity. Then, an exploratory analysis of the metabolite profile of the most successful fraction was performed by gas chromatography-mass spectrometry (GC-MS). Among the dichloromethane soluble fractions derived from *Annona cacans* Warm., *Annona coriacea* Mart., *Annona neolaurifolia* H. Rainer, *Annona sylvatica* A.St.-Hil., *Duguetia lanceolata* A.St.-Hil., *Guatteria australis* A.St.-Hil., *Xylopia brasiliensis* Spreng., *Xylopia emarginata* Mart. and *Xylopia sericea* A.St.-Hil., only the fraction from *D. lanceolata* stem bark reduced the survival of *T. tumidus* females. However, ovicidal activity was not detected when *D. lanceolata* stem bark was evaluated against *T. tumidus* eggs. Further, we studied the effect of dichloromethane soluble fractions from *D. lanceolata* leaves, berry fruits and stem bark on *T. urticae*, and the stem bark was found to be the most active fraction against *T. urticae*. The metabolite profile analysis of *D. lanceolata* stem bark by GC-MS, suggested that the main constituents were 2,4,5-trimethoxystyrene and trans-asarone.

Key words: Botanic acaricide, secondary metabolites, 1,2,4-trimethoxy-5-vinylbenzene, 1,2,4-trimethoxy-5-[(1E)-1-propen-1-yl]benzene

Resumo

As espécies do gênero *Tetranychus* alimentam-se de tecidos vegetais acarretando redução na capacidade fotossintética da planta, que pode resultar em morte dos tecidos vegetais, como consequência são

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consideráveis as perdas na produção causadas por esses artrópodes. Assim, com vistas a contribuir para o desenvolvimento de novos produtos para o controle dos ácaros *Tetranychus tumidus* Banks e *Tetranychus urticae* Koch (Acari: Tetranychidae) o objetivo inicial deste trabalho foi selecionar frações solúveis em diclorometano com atividade acaricida, provenientes de extratos metanólicos de espécies de anonáceas. Em seguida, buscou-se realizar análise exploratória do perfil metabólito da fração selecionada por meio da cromatografia gasosa acoplada a espectrometria de massas (CG-EM). Dentre as frações solúveis em diclorometano oriundas de *Annona cacans* Warm., *Annona coriacea* Mart., *Annona neolaurifolia* H. Rainer, *Annona sylvatica* A.St.-Hil., *Duguetia lanceolata* A. St.-Hil., *Guatteria australis* A.St.-Hil., *Xylopia brasiliensis* Spreng., *Xypolia emarginata* Mart. e *Xylopia sericea* A.St.-Hil., apenas aquela proveniente das cascas do caule de *D. lanceolata* causou redução na sobrevivência de fêmeas de *T. tumidus*. Entretanto, quando avaliado o efeito da fração das cascas do caule de *D. lanceolata* sobre ovos de *T. tumidus* não foi constatada atividade ovicida. Quanto a *T. urticae*, foi estudado o efeito das frações solúveis em diclorometano provenientes das folhas, frutos e cascas do caule de *D. lanceolata*, sendo que a mais ativa contra o ácaro foi a proveniente das cascas do caule. A análise do perfil metabólito da fração das cascas do caule de *D. lanceolata*, por meio de CG-MS, sugeriu que os constituintes majoritários são o 2,4,5-trimetoxiestireno e a *trans*-asarona.
Palavras-chave: Acaricida botânico, metabólitos secundários, 1,2,4-trimetoxi-5-vinilbenzeno, 1,2,4-trimetoxi-5-[(E)-prop-1-enil]benzeno

Introduction

The Tetranychidae family includes the main group of phytophagous mites. Several species of this family are pests in many cropping systems (MARCIC, 2012). Among the species of phytophagous mites that cause agricultural losses, *Tetranychus tumidus* Banks (Acari: Tetranychidae) is the key pest in plantain (*Musa* spp.) nurseries in Cuba, whereas *Tetranychus urticae* Koch (Acari: Tetranychidae) is an important pest of many horticultural crops in both Brazil and Cuba. Synthetic acaricides have been used as the main mite control strategy. However, the indiscriminate use of these pesticides, coupled with the high reproductive potential and short life cycle of the mites, has led to the development of resistant populations in different places (SATO et al., 2005; LEEUWEN et al., 2010; SOKOLYANSKAYA, 2010).

In this context, the use of derivatives from plant metabolism to control mites has been the subject of an increasing number of studies (YANAR et al., 2011; ERDOGAN et al., 2012; VERONEZ et al., 2012). Acaricidal activity was previously reported in an extract of the roots of *Phytolacca americana* L. (Phytolaccaceae) (DING et al., 2013). Similarly, leaf extracts from *Anisosciadium orientale* DC. (Apiaceae), *Scaligeria meifolia* Boiss. (Apiaceae), *Trigonella elliptica* Boiss. (Fabaceae), and *Dodonaea viscosa* L. (Sapindaceae) showed promising acaricidal activity (GHADERI et al., 2013). It is also possible that *Deverra scoparia* Coss. & Durieu (Apiaceae), *Haplophyllum tuberculatum* (Forsskal) A. Juss. (Rutaceae), *Chrysanthemum coronarium* L. (Asteraceae), and *Mentha pulegium* L. (Lamiaceae) extracts have acaricidal activity (ATTIA et al., 2012).

It has been shown that plant metabolites can have repellent effects, contact toxicity, inhibit enzymatic processes, and change reproductive variables, which kill or reduce the reproductive performance of mites (LÜMMEN, 1998; LABORDA et al., 2013; ZHANG et al., 2013). Numerous plant families are known to produce metabolites that are toxic to these phytophagous mites, among which, the Annonaceae family deserves special attention, because there have been many intensive studies that have evaluated this family's use as a pesticide producers (MCLAUGHLIN, 2008; GUPTA et al., 2011; MADHUMITHA et al., 2012). This study aimed to evaluate the bioactivity of dichloromethane soluble fractions from methanol extracts of plants from the Annonaceae family, originating from the Alto do Rio Grande region in Minas Gerais, Brazil,

against a Cuban population of *T. tumidus*. *Duguetia lanceolata* fractions, the most active plant fractions against *T. tumidus*, were tested to see if they could control *T. urticae*. We also analyzed the metabolic profile of *D. lanceolata* by gas chromatography-mass spectrometry (GC-MS).

Materials and Methods

Collection and processing of plant materials

The botanical materials used to perform the bioassays were collected from the Alto Rio Grande region-Lavras/Minas Gerais, between March and April 2011. Samples of the materials were also used for making voucher specimens, which were deposited in the ESAL (Escola Superior de Agricultura de Lavras) herbarium (Table 1). The leaves, stem bark, and berry fruits were initially dried in an oven with forced ventilation at 40°C for 48, 72, or 96 h. Then the samples were ground in a Willey mill, which produced the ground materials that were used in the preparation of the extracts and fractions.

Table 1. Botanical materials used in the preparation of dichloromethane soluble fractions derived from plant extracts.

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Scientific name	Synonymies[1]	Collected part	Voucher specimen number
Annona cacans Warm.	-	Leaves	27639
Annona coriacea Mart.	-	Leaves	27640
Annona neolaurifolia H. Rainer	*Rollinia laurifolia* Schltd	Leaves Stem bark	27638
Annona sylvatica A.St.-Hil.	*Annona silvestris* Vell., *Annona fagifolia* A.St.-Hil. & Tul., *Annona exalbida* Vell., *Rollinia sylvatica* (A.St.-Hil.) Mart., *Rollinia exalbida* (Vell.) Mart., *Rollinia fagifolia* A.St.-Hil.	Leaves	27647
Duguetia lanceolata A.St.-Hil.	-	Leaves Berry fruits Stem bark	27631
Guatteria australis A.St.-Hil.	*Guatteria acutiflora* Mart., *Guatteria acutipetala* R.E.Fr., *Guatteria asterantha* R.E.Fr., *Guatteria blanchetiana* R.E.Fr., *Guatteria clavigera* R.E.Fr., *Guatteria curvinervia* R.E.Fr., *Guatteria densicoma* Mart., *Guatteria dimorphopetala* R.E.Fr., *Guatteria dusenii* R.E.Fr., *Guatteria flava* A.St.-Hil., *Guatteria fruticosa* R.E.Fr., *Guatteria glabrescens* R.E.Fr., *Guatteria gomeziana* A.St.-Hil., *Guatteria hilariana* Schltdl., *Guatteria hookeri* A.St.-Hil. & Tul., *Guatteria klotzschiana* Mart., *Guatteria lutea* A.St.-Hil., *Guatteria minarum* R.E.Fr., *Guatteria mosenii* R.E.Fr., *Guatteria neglecta* R.E.Fr., *Guatteria nigrescens* Mart., *Guatteria odontopetala* Mart., *Guatteria paranensis* R.E.Fr., *Guatteria parvifolia* R.E.Fr., *Guatteria penduliflora* R.E.Fr., *Guatteria polycarpa* R.E.Fr., *Guatteria psilopus* Mart., *Guatteria reflexa* R.E.Fr., *Guatteria riedeliana* R.E.Fr., *Guatteria salicifolia* R.E.Fr., *Guatteria silvatica* R.E.Fr., *Guatteria sordida* R.E.Fr., *Guatteria tenuis* R.E.Fr.	Leaves	27645

... Continuation

Xylopia brasiliensis Spreng.	*Xylopia gracilis* R.E.Fr.	Leaves	27636
Xylopia emarginata Mart.	-	Stem bark Leaves	-
Xylopia sericea A.St.-Hil.	*Xylopia intermedia* R.E.Fr.	Stem bark Leaves Berry fruits	27646

[1] Oliveira-Filho (2013).

Preparation of plant extracts and liquid-liquid partitioning

The dried and ground plant material (50 g) was immersed in methanol (300 mL) for 24 h. Then the mixtures were filtered through hydrophilic cotton and 200 mL methanol was added to the residue. The extraction procedure was repeated eight times. The liquid phases were combined and the solvent removed in a rotary evaporator, which resulted in the dried methanolic plant extracts. The methanolic plant extracts (5 g) were suspended in water (20 mL) so that they could be liquid-liquid partitioned with dichloromethane (3 × 20 mL). The water-soluble phase was discarded in each extract, and the phases that were soluble in dichloromethane were combined, resulting in a solution that was then treated with anhydrous Na_2SO_4 to remove any humidity. The mixture was filtered through cotton, and concentrated and dried in a rotary evaporator. The final residue was lyophilized to produce the final dichloromethane soluble fraction (ÁLVAREZ-COLOM et al., 2007, 2008, 2009). The different fractions were transported from Brazil to Cuba, which required a Plant Import Permit. This was issued by IBAMA-Instituto Brasileiro de Meio Ambiente (license number: 12BR008914/DF).

Rearing and maintenance of the mites

The mites used in the bioassays were obtained from the Acarology Laboratory at CENSA (Centro Nacional de Sanidad Agropecuaria, San José de las Lajas, Cuba), where specimens of *T. tumidus* were maintained on plantain leaves (*Musa* sp. var. Ciento en boca). The *T. urticae* were maintained on citrus leaves (*Citrus latifolia*). Adult females, aged between 48 and 72 h, were used to conduct the bioassays.

Effects of dichloromethane soluble fractions on T. tumidus adult females

The dichloromethane soluble fractions were obtained from methanolic extracts derived from the leaves of *Annona cacans* Warm., *Annona coriacea* Mart., *Annona sylvatica* A.St.-Hil., *Guatteria australis* A.St.-Hil., and *Xylopia brasiliensis* Spreng.; the leaves and stem bark of *Annona neolaurifolia* H. Rainere and *Xylopia emarginata* Mart.*;* and the leaves, stem bark, and berry fruits of *Duguetia lanceolata* A.St.-Hil. and *Xylopia sericea* A.St.-Hil**.** These fractions (10 mg) were mixed with DMSO (50 µL), and then an aqueous solution of Tween 80 (0.01 v v^{-1}) (950 µL) was added, which resulted in a sample with a final volume of 1.0 mL and a concentration of 10 mg mL^{-1}.

A microimmersion bioassay, adapted from Dennehy et al. (1993), was used for the topical application of dichloromethane soluble fractions to *T. tumidus* females. Plastic pipette tips (200 µL) were cut in half and reconnected, so that a piece of organza fabric could be inserted to separate the internal space of the tip into two parts. A hose was attached to the thicker end of the tip, and at least

25 adult females were suctioned up. Then, the tip was connected to a micropipette and 100 µL of the fraction solution in DMSO and Tween 80 were sucked onto the tip. The mites remained immersed for 30 s and afterward were transferred to filter paper (2 cm × 2 cm) to remove the treatments that had previously been in contact with the mites. Then, the mites were transferred to pieces of plantain leaves (approximately 5 cm × 3 cm) that were surrounded by cotton moistened with water. These inoculated leaves were then placed inside a Petri dish, which corresponds to an experimental plot.

The experiment had a completely randomized design with four replications per treatment. An experimental plot consisted of at least 25 adult females. Water and an aqueous solution containing DMSO (0.05 v v^{-1}) and Tween 80 (0.01 v v^{-1}) were used as negative controls. The positive control was the acaricide, dicofol (0.01 v v^{-1}) (Dicofol 18,5 CE). The evaluations were performed 24, 48, and 72 h after the beginning of the experiment by counting the number of live and dead mites.

Effect of the dichloromethane soluble fraction from D. lanceolata stem bark on T. tumidus eggs

The treatments used were water; DMSO (0.05 v v^{-1}) + Tween 80 (0.01 v v^{-1}); soluble fraction from *D. lanceolata* stem bark (10 mg mL^{-1}), and dicofol (0.01 v v^{-1}) (Dicofol 18,5 CE). Plantain leaf pieces (3.0 × 3.0 cm), with the edges wrapped in cotton moistened with distilled water, were placed in Petri dishes. A total of 100 females, aged between 24 and 48 h, were transferred to each piece of leaf. They were kept there for 24 h so that they could lay eggs. The females were then removed and the eggs were counted. Excess eggs were removed with a paintbrush, which left approximately 50 eggs on each piece of leaf. The leaf pieces containing the eggs were immersed in 4 mL of each treatment for 30 seconds. Then the leaves were maintained for approximately 2 h at room conditions to allow them to dry out and then were arranged in Petri dishes (one piece of leaf per dish). The nymphs were counted after six days of treatment.

The experimental design was completely randomized and each experimental plot corresponded to a Petri dish with a piece of leaf containing about 50 eggs. Four replications were used per treatment, and the experiment was repeated twice.

Effect of the D. lanceolata stem bark fraction on T. urticae adult females

This bioassay used *T. urticae* females that had been kept in the laboratory. The method used was the adapted microimmersion bioassay, described above. The treatments were the fractions from the leaves, stem barks and berry fruits of *D. lanceolata* (10 mg mL^{-1}). The negative controls were water and DMSO (0.05 v v^{-1}) + Tween 80 (0.01 v v^{-1}) and the positive control was dicofol (0.01 v v^{-1}) (Dicofol 18,5 CE). The experimental plot was a Petri dish containing a piece of citrus leaf and 25 females. The experimental design was completely randomized, and the evaluations were performed 24, 48, and 72 h after the experiment had begun by counting the number of live and dead mites. The experiment was repeated twice. In the first experiment, five replicates were used, whereas the second experiment only contained four replicates per treatment.

Analysis by GC-MS

The GC-MS analyses were performed on a gas chromatograph coupled to a mass spectrometer (Shimadzu GCMS-QP2010 Plus), equipped with an AOC-10 automatic injection system. Analyses were conducted using an Rxi-1 capillary column (30 m × 0.25 mm), coated with 100% polydimethylsiloxane. The thickness of the film was 0.25 µm, and helium was used as the carrier gas (flow rate of 7.2 $mL \cdot min^{-1}$). The injector temperature was 250°C, while the

column temperature ranged from 150°C to 280°C at a rate of 3°C·min^{-1}. The injection volume was 1 μL in split mode at a rate of 10:1. MS analysis was performed using a quadrupole system (QP-2010plus) with electron impact ionization at 70 eV under the same conditions as described above. The structures of the major compounds were suggested by comparing them to the structures found in the NIST 08 mass spectra libraries and in the literature.

Statistical analysis

In order to verify that the data was normal, the collected data were subjected to the Shapiro-Wilk test, using the mvnormtest package (JAREK, 2009). Analysis of variance was undertaken, and the means were compared by the Scott-Knott test, using the Laércio statistical package (SILVA, 2010). For the experiments that were repeated, joint analysis of data was conducted and the Bartlett test was used to verify the homogeneity of variances (R DEVELOPMENT CORE TEAM, 2013). R software, version 3.01 (R DEVELOPMENT CORE TEAM, 2013) was used to perform the analyses.

Results and Discussion

Effects of dichloromethane soluble fractions on T. tumidus adult females

Only the dichloromethane soluble fraction of *D. lanceolata* stem bark reduced the survival of *T. tumidus* females after 24 h of immersion. In the evaluations performed after 48 h and 72 h, it was observed that the fractions originating from the *D. lanceolata* stem bark and *X. sericea* berry fruits resulted in a lower average *T. tumidus* survival, when compared to the negative controls ($F = 123.34$; $df = 17$; $p \leq 0.01$) (Table 2).

The *D. lanceolata* stem bark fraction was the most active against *T. tumidus* females. However, the fractions from leaves and fruit extracts were not bioactive. This result was possibly due to differences in intra-plant metabolite allocations (SOMKUWAR; KAMBLE, 2013; UDDIN et al., 2013). The stem bark results are the first report of acaricidal activity by *D. lanceolata* extracts. Although studies using *Duguetia* spp. are scarce, it is noteworthy that the *Duguetia furfuracea* (St. Hil) Bent. extract was not active against *Rhipicephalus microplus* Canestrini (Acari: Ixodidae) (VALENTE et al., 2014). This study seems to confirm the results from previous phytochemical studies with *D. lanceolata*, which also detected chemical groups that possessed acaricidal activity (NEVES; DA CAMARA, 2011), such as isoquinoline alkaloids (FISCHER et al., 2004; TEMPONE et al., 2005) and sesquiterpenes (SOUSA et al., 2012).

Previous studies have suggested that *Annona* spp. has acaricidal activity against *Tetranychus* spp. (POTENZA et al., 2006; LIN et al., 2009), which in part may be due to the production of acetogenins by this species, which are known to have acaricidal activity (LÜMMEN, 1998). Although there are no previous reports of *A. cacans* acaricide activity against *Tetranychus* spp., alkaloids have been isolated from this plant, such as liriodenine (SAITO; ALVARENGA, 1994), for which an acaricidal activity against *Dermatophagoides pteronyssinus* (Trouessart) (Acari: Pyroglyphidae) has already been reported (AKENDENGUE et al., 2003). However, in this study, the *Annona* genus species were not active against *T. tumidus*.

Table 2. Survival (%) (mean ± SEM) of *Tetranychus tumidus* females subjected to treatment with soluble dichloromethane fractions from Annonaceae methanolic extracts, using an adapted micro-immersion bioassay technique, after 24, 48, and 72 h.

Treatments	n[1]	24 h[2]	48 h[2]	72 h[2]
Water	148	93.6 ± 2.96 aA	92.7 ± 2.40 aA	91.8 ± 2.10 aA
DMSO (0.05 v·v^{-1}) + Tween 80 (0.01 v·v^{-1})	139	94.0 ± 2.15 aA	94.0 ± 2.15 aA	94.0 ± 2.15 aA
Annona cacans leaves	177	97.2 ± 1.05 aA	97.2 ± 1.05 aA	96.0 ± 1.11 aA
Annona coriacea leaves	161	93.8 ± 3.64 aA	92.9 ± 4.05 aA	92.9 ± 4.05 aA
Annona neolaurifolia leaves	137	93.2 ± 1.04 aA	93.2 ± 1.04 aA	93.2 ± 1.04 aA
Annona neolaurifolia stem bark	160	94.2 ± 2.60 aA	94.2 ± 2.60 aA	93.6 ± 2.18 aA
Annona sylvatica leaves	123	89.7 ± 4.78 aA	89.7 ± 4.78 aA	87.4 ± 4.39 aA
Duguetia lanceolata stem bark	142	68.4 ± 6.24 bA	62.6 ± 8.19 cA	59.0 ± 7.43 cA
Duguetia lanceolata leaves	121	91.0 ± 1.90 aA	90.0 ± 1.68 aA	88.8 ± 1.84 aA
Duguetia lanceolata berry fruits	170	93.3 ± 0.82 aA	93.3 ± 0.82 aA	92.1 ± 0.87aA
Guatteria australis leaves	143	93.5 ± 2.56 aA	88.9 ± 5.16 aA	88.9 ± 5.16 aA
Xylopia brasiliensis leaves	166	96.8 ± 1.24 aA	96.8 ± 1.24 aA	96.8 ± 1.24 aA
Xylopia emarginata stem bark	181	95.2 ± 2.82 aA	95.2 ± 2.82 aA	95.2 ± 2.82 aA
Xylopia emarginata leaves	146	90.0 ± 2.96 aA	90.0 ± 2.96 aA	88.4 ± 4.52aA
Xylopia sericea stem bark	178	95.8 ± 2.38 aA	95.8 ± 2.38 aA	94.4 ± 1.94 aA
Xylopia sericea leaves	177	94.6 ± 3.59 aA	93.6 ± 3.20 aA	93.6 ± 3.20 aA
Xylopia sericea berry fruits	105	84.3 ± 4.67 aA	78.5 ± 8.29 bA	76.7 ± 8.42 bA
Dicofol at 0.01 (v·v^{-1})	113	0.0 ± 0.00 cA	0.0 ± 0.00 dA	0.0 ± 0.00 dA

[1]Number of treated females; [2] Values with the same letters, uppercase in rows and lowercase in columns, do not differ according to the Scott-Knott test ($P \leq 0.01$). Coefficient of variation (CV): 8.29%.

Similarly, no previous reports of acaricidal activity were found for *A. coriacea, A. neolaurifolia*, and *A. sylvatica*, despite reports in the literature on the extraction of substances from these plants that may have potential uses as pesticides. For example, acetogenin and gigantetronenin were isolated from *A. coriacea* roots (SILVA et al., 1996), and these compounds inhibit the mitochondrial complex I (ÁLVAREZ-COLOM et al., 2009). The soluble methanol and hexane extracts from *A. coriacea* seeds are also known to have high insecticidal activities (COSTA et al., 2013). The differences between the results obtained in this study and those reported in the literature may be due to the use of different methodologies to obtain the metabolites.

No previous reports on acaricide activity were found for *A. neolaurifolia* and *A. sylvatica*. However, phytochemical studies have indicated that these species produce substances that may have pesticidal activity (NASCIMENTO et al., 2003; PIMENTA et al., 2005; FORMAGIO et al., 2013). Acetylcholinesterase inhibitory activity has been reported for the alcoholic extract from the branches of *G. australis*, while the same bioactivity was not observed for the leaf extract (CARDOSO-LOPES et al., 2008). This last result is consistent with the inactivity of the *G. australis* leaf fraction observed in the present study.

For the species from the *Xylopia* genus, just a fraction from *X. sericea* fruit caused a reduction in *T. tumidus*. Previous studies on the essential oils from leaves and fruits of this plant have demonstrated that they have an acaricidal effect on *T. urticae* (PONTES et al., 2007). No previous reports of acaricide activity were found for *X. brasiliensis* and *X. emarginata*. However, phytochemical studies have indicated that metabolites from these plants may have toxic effects on herbivores (LAGO et al., 2003, 2005; MOREIRA et al., 2013).

Effect of the dichloromethane soluble fraction from D. lanceolata stem bark on T. tumidus eggs

There was no reduction in the number of hatched nymphs, compared to the negative controls ($F = 17.14$; $df = 3$; $p \leq 0.01$), when *T. tumidus* eggs were treated with the *D. lanceolata* stem bark fraction (Table 3). This may be because the egg stage is the most resistant to adverse environmental conditions in many groups of arthropods. Therefore, natural plant products are generally more toxic to larval and adult stages of *Tetranychus* spp. (CHANDRASHEKHARAIAH et al., 2011; ERDOGAN et al., 2012).

Table 3. Numbers of hatched *Tetranychus tumidus* nymphs (%) (mean ± SE) after egg treatment with the dichloromethane soluble fraction from *Duguetia lanceolata* stem bark.

Treatment	n[1]	Nymphs hatched (%) ± SE[2]
Water	408	82.9 ± 9.21 a
DMSO (0.05 v·v^{-1}) + Tween 80 (0.01 v·v^{-1})	385	77.6 ± 11.93 a
Duguetia lanceolata stem bark	432	71.7 ± 9.34 a
Dicofol®	298	0.0 ± 0.00 b

[1]Total number of eggs; [2]Values with the same letters do not differ according to the Scott-Knott test ($P \leq 0.01$). Coefficient of variation (CV): 46.48 %.

Effect of the D. lanceolata stem bark fraction on T. urticae adult females

The *D. lanceolata* stem bark fraction was most active against *T. tumidus*, so the fractions from the plant tissues of this plant were selected for the *T. urticae* bioassay. It was found that the leaf, berry fruit, and stem bark fractions reduced *T. urticae* survival ($F = 648.78$; $df = 5$; $p \leq 0.01$). Survival was not affected over the period of exposure to treatments ($F = 1.256$; $df = 2$; $p = 0.28$). However, the best results were found when the *D. lanceolata* stem bark fraction was applied which, 72 h after the start of the bioassay, caused 95.5% mortality, a value statistically equal to that observed for the positive control, dicofol (Table 4). The *D. lanceolata* stem bark fraction was more active against *T. urticae* than *T. tumidus*, which must be a result of species-specific variations. It is relatively common that related species of arthropods have different abilities and mechanisms for detoxifying toxic metabolites (KIM et al., 2003; SONG et al., 2011).

Table 4. Survival (%) (mean ± SEM) of *Tetranychus urticae* females subjected to treatment with soluble dichloromethane fractions from Annonaceae methanolic extracts, using an adapted micro-immersion bioassay technique, after 24, 48 and 72 h.

Treatment	n[1]	24 h[2]	48 h[2]	72 h[2]
Water	193	90.4 ± 1.89 aA	88.3 ± 1.84 aA	87.6 ± 1.81 aA
DMSO + Tween	269	89.9 ± 0.99 aA	86.8 ± 1.83 aA	85.3 ± 1.99 aA
Duguetia lanceolata leaves	233	83.4 ± 2.69 bA	81.1 ± 3.62 bA	80.6 ± 3.49 bA
Duguetia lanceolata berry fruits	257	77.5 ± 3.64 bA	75.7 ± 2.67 bA	74.3 ± 2.67 bA
Duguetia lanceolata stem barks	243	5.9 ± 4.39 cA	4.5 ± 4.28 cA	4.5 ± 4.28 cA
Dicofol	257	0.0 ± 0.00 cA	0.0 ± 0.00 cA	0.0 ± 0.00 cA

[1]Total number of eggs; [2]Values with the same letters, uppercase in rows and lowercase in columns, do not differ according to the Scott-Knott test ($P \leq 0.01$). Coefficient of variation (CV): 14.62%.

Analysis by GC-MS

The constituent analysis of the *D. lanceolata* stem bark fraction revealed the presence of two major constituents. The first peak showed a retention time (RT) of 4.28 min, and the highest mass/charge value (m/z) was equal to 194 u, which possibly corresponded to the molecular ion peak $[M]^{+\cdot}$. Studies previously performed by our research group (ALVES, 2014), and comparison with the literature data (Table 5), suggested that this substance was 2,4,5-trimethoxystyrene (RT = 4.28 min) (Figure 1). The other substance had a RT equal to 5.74 minutes, and the highest m/z value was 209, which probably corresponded to $[M]^{+}$. A comparison of its mass spectra with mass spectra in the NIST library 08 suggested that this compound was the phenylpropanoid compound, trans-asarone (Figure 1), with similarity rates equal to 96%. This result was corroborated by comparison of the mass spectra obtained in this study with those described in the literature for this substance (Table 5).

Table 5. Mass/charge (m/z) ratio for the peaks observed in the mass spectra of the major compounds (2,4,5-trimethoxystyrene and trans-asarone) in the chromatogram of the *Duguetia lanceolata* stem bark fraction. The analyses were performed by mass spectrometry coupled to gas chromatography.

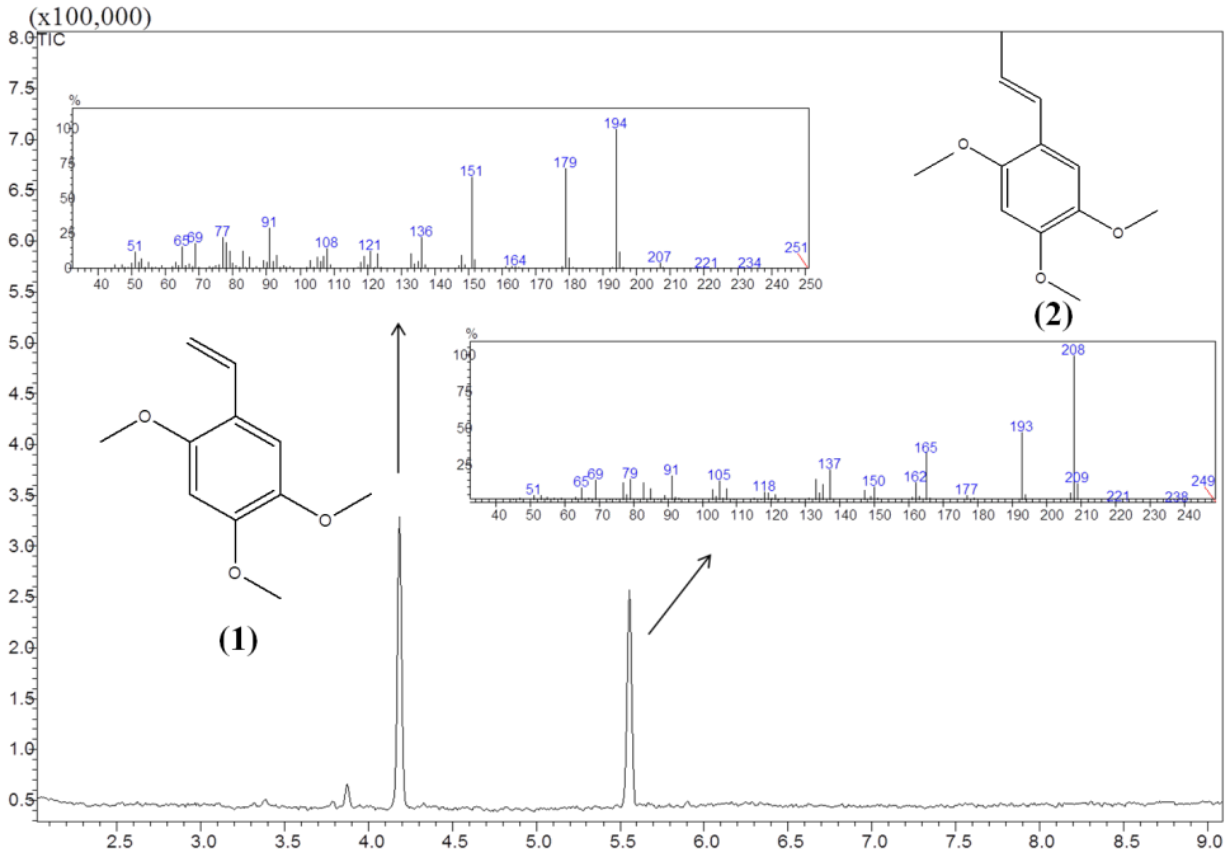
2,4,5-trimethoxystyrene				trans-asarone					
m/z[1]	IR[1,2]	(m/z)[3]	IR[2,3]	m/z[1]	IR[1,2]	(m/z)[4]	IR[2,4]	(m/z)[5]	IR[2,5]
194	100	194	100	208	100	208	100	208	100
179	71	179	61	193	47	193	45	193	38
151	65	151	46	177	6	177	4	177	4
136	23	136	13	165	33	165	25	165	23
121	11	121	7	162	14	162	13	-	-
108	11	-	-	150	11	150	9	150	8
91	28	91	11	137	22	137	9	137	8
77	22	-	-	118	5	-	-	-	-
69	17	69	11	-	-	119	9	-	-
65	14	-	-	105	14	105	8	105	4
51	11	51	8	91	17	91	11	91	8
-	-	39	4	79	14	-	-	79	4
				-	-	77	8	-	-
				69	14	69	13	69	8
				65	8	-	-	-	-
				51	3	-	-	-	-

[1]Experimental value; the substances were ionized by electron impact at 70 eV; [2]RPI = relative peak intensity; [3]Nagashima et al. (1999); [4]Oprean et al. (1998); [5]Zuo et al. (2012).

Trans-asarone and 2,4,5-trimethoxystyrene are relatively common metabolites in the Annonaceae family (WANG et al., 1988; NGADJUI et al., 1989; SILVA et al., 2007; YAPI et al., 2012). It is noteworthy that trans-asarone was recently identified as a component of an essential oil extracted from *D. lanceolata* branches (SOUSA et al., 2012). Moreover, the abundance of 2,4,5-trimethoxystyrene and trans-asarone in the *D. lanceolata* stem bark, as shown by the GC-MS analysis (Figure 1), is consistent with the high activity against *T. urticae*, since both molecules are known to have insecticidal activity (KOONA; BOUDA, 2006; BHARDWAJ et al., 2010). It is noteworthy that the acaricidal

activity of these molecules is supported by the fact that other molecules, also belonging to the phenylpropanoids chemical class, have also been reported to have acaricidal activity. In particular, the phenylpropanoid, eugenol, is known to have activity against *T. urticae*, and some researchers have attributed its mechanism of action and toxicity to fumigant contact (HAN et al., 2011; ARAÚJO et al., 2012).

Figure 1. Chromatogram of the dichloromethane soluble fraction from the methanolic extract of *Duguetia lanceolata* stem barks, which was obtained by gas chromatography coupled to mass spectrometry. The main substances, identified as 2,4,5-trimethoxystyrene (1) and trans-asarone (2), were ionized by electron impact at 70 eV.



Conclusions

The fraction from *D. lanceolata* stem barks reduced *T. tumidus* and *T. urticae* survival. The highest mortality rate was observed for *T. urticae*. According to the GC-MS analysis, the major constituents of the dichloromethane soluble fraction in the extract from *D. lanceolata* were 2,4,5-trimethoxystyrene and trans-asarone, of which there are no previous reports regarding their pesticide activity. Therefore, the results of this study show that *D. lanceolata* stem bark could potentially be used to develop new products for the control of *T. urticae* and *T. tumidus*.

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References

AKENDENGUE, B.; NGOU-MILAMA, E.; BOUROBOU-BOUROBOU, H.; ESSOUMA, J.; ROBLOT, F.; GLEYE, C.; LAURENS, A.; HOCQUEMILLER, R.; LOISEAU, P.; BORIES, C. Acaricidal activity of *Uvaria versicolor* and *Uvaria klaineana* (Annonaceae). *Phytotherapy Research*, London, v. 17, n. 4, p. 364- 367, 2003.

ÁLVAREZ-COLOM, O.; BARRACHINA, I.; MINGOL, I. A.; MAS, M. C. G.; SANZ, P. M.; NESKE, A.; BARDON, A. Toxic effects of annonaceous acetogenins on *Oncopeltus fasciatus*. *Journal of Pest Science*, New York, v. 81, n. 2, p. 85-89, 2008.

ÁLVAREZ-COLOM, O.; NESKE, A.; CHAHBOUNE, N.; ZAFRA-POLO, M. C.; BARDÓN, A. Tucupentol, a novel mono-tetrahydrofuranic acetogenin from *Annona montana*, as a potent inhibitor of mitochondrial complex I. *Chemistry & Biodiversity*, Weinheim, v. 6, n. 3, p. 335-340, 2009.

ÁLVAREZ-COLOM, O.; NESKE, A.; POPICH, S.; BARDÓN, A. Toxic effects of annonaceous acetogenins from *Annona cherimolia* (Magnoliales: Annonaceae) on *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Journal of Pest Science*, New York, v. 80, n. 1, p. 63-67, 2007.

ALVES, D. S. *Prospecção de metabólicos de anonáceas ativos para Spodoptera frugiperda e Tetranychus_spp*. 2014. Tese (Doutorado em Agronomia/Entomologia) - Universidade Federal de Lavras, Lavras.

ARAÚJO, M. J. C.; CÂMARA, C. A. G.; BORN, F. S.; MORAES, M. M.; BADJI, C. A. Acaricidal activity and repellency of essential oil from *Piper aduncum* and its components against *Tetranychus urticae*. *Experimental and Applied Acarology*, Amsterdam, v. 57, n. 2, p. 139-155, 2012.

ATTIA, S.; GRISSA, K. L.; GHRABI, Z. G.; MAILLEUX, A. C.; LOGNAY, G.; HANCE, T. Acaricidal activity of 31 essential oils extracted from plants collected in Tunisia. *Journal of Essential Oil Research*, Carol Stream, v. 24, n. 3, p. 279-288, 2012.

BHARDWAJ, A.; TEWARY, D. K.; KUMAR, R.; KUMAR, V.; SINHA, A. K.; SHANKER, A. Larvicidal and structure-activity studies of natural phenylpropanoids and their semisynthetic derivatives against the tobacco armyworm *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae). *Chemistry & Biodiversity*, Weinheim, v. 7, n. 1, p. 168-177, 2010.

CARDOSO-LOPES, E. M.; CARREIRA, R. C.; AGRIPINO, D. G.; TORRES, L. M. B.; CORDEIRO, I.; BOLZANI, V. D. S.; DIETRICH, S. M. D. C.; YOUNG, M. C. M. Screening for antifungal, DNA-damaging and anticholinesterasic activities of Brazilian plants from the Atlantic Rainforest - Ilha do Cardoso State Park. *Brazilian Journal of Pharmacognosy*, João Pessoa, v. 18, p. 655-660, 2008. Supplement.

CHANDRASHEKHARAIAH; SANNAVEERAPPANAVAR, V. T.; PRASHANT; SATHISH; NAVEENA, N. L. Evaluation of acaricidal properties of selected plant extracts and organic materials against two spotted spider mite, *Tetranychus urticae* Koch (Tetranychidae: Acari). *Biopesticides International*, Jalandhar, v. 7, n. 1, p. 35-42, 2011.

COSTA, M. S.; PEREIRA, M. J. B.; OLIVEIRA, S. S.; SOUZA, P. T.; DALL'OGLIO, E. L.; ALVES, T. C. Anonáceas provocam mortalidade em larvas de *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae). *Revista Brasileira de Biociências*, Porto Alegre, v. 11, n. 2, p. 184-190, 2013.

DENNEHY, T.; FARNHAM, A.; DENHOLM, I. The microimmersion bioassay: a novel method for the topical application of pesticides to spider mites. *Pesticide Science*, Oxford, v. 39, n. 1, p. 47-54, 1993.

DING, L. J.; DING, W.; ZHANG, Y. Q.; LUO, J. X. Bioguided fractionation and isolation of esculentoside P from *Phytolacca americana* L. *Industrial Crops and Products*, New York, v. 44, p. 534-541, 2013.

ERDOGAN, P.; YILDIRIM, A.; SEVER, B. Investigations on the effects of five different plant extracts on the two-spotted mite *Tetranychus urticae* Koch (Arachnida: Tetranychidae). *Psyche: Journal of Entomology,* Cambridge, v. 2012, art. nº. 125284, p. 1-5, 2012.

FISCHER, D. C. H.; GUALDA, N. C. D. A.; BACHIEGA, D.; CARVALHO, C. S.; LUPO, F. N.; BONOTTO, S. V.; ALVES, M. D. O.; YOGI, A.; SANTI, S. M. D.; AVILA, P. E.; KIRCHGATTER, K; MORENO, P. R. H. In vitro screening for antiplasmodial activity of isoquinoline alkaloids from Brazilian plant species. *Acta Tropica*, Basel, v. 92, n. 3, p. 261-266, 2004.

FORMAGIO, A. S. N.; VIEIRA, M. D. C.; DOS SANTOS, L. A. C.; CARDOSO, C. A. L.; FOGLIO, M. A.; CARVALHO, J. E. de; ANDRADE-SILVA, M.; KASSUYA, C. A. L. Composition and evaluation of the anti-inflammatory and anticancer activities of the essential oil from *Annona sylvatica* A. St. -Hil. *Journal of Medicinal Food*, Florida, v. 16, n. 1, p. 20-25, 2013.

GHADERI, S.; MINAEI, K.; ROWSHAN, V.; GHAMADYARI, M. Toxicity and ovicidal activity of different plant extracts on two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae). *Archives of Phytopathology and Plant Protection*, London, v. 46, n. 1, p. 120-126, 2013.

GUPTA, A.; PANDEY, S.; SHAH, D. R.; YADAV, J. S.; SETH, N. R. Annonaceous acetogenins: the unrevealed area for cytotoxic and pesticidal activities. *Systematic Reviews in Pharmacy*, Mumbai, v. 2, n. 2, p. 104-109, 2011.

HAN, J.; KIM, S. I.; CHOI, B. R.; LEE, S. G.; AHN, Y. J. Fumigant toxicity of lemon eucalyptus oil constituents to acaricide-susceptible and acaricide-resistant *Tetranychus urticae*. *Pest Management Science,* Sussex, v. 67, n. 12, p. 1583-1588, 2011.

JAREK, S. *R Package*: mvnormtest: normality test for multivariate variables. 2009. Available at: <http://cran.r-project.org/web/packages/mvnormtest/index>. Accessed at: 10 dec. 2013.

KIM, E. H.; KIM, H. K.; AHN, Y. J. Acaricidal activity of clove bud oil compounds against *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (Acari: Pyrogiyphidae). *Journal of Agricultural and Food Chemistry*, Easton, v. 51, n. 4, p. 885-889, 2003.

KOONA, P.; BOUDA, H. Biological Activity of *Pachypodanthium staudtii* (Annonaceae) against the bean beetle *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae). *Journal of Applied Sciences Research*, Faisalabad, v. 2, n. 12, p. 1129-1131, 2006.

LABORDA, R.; MANZANO, I.; GAMÓN, M.; GAVIDIA, I.; PÉREZ-BERMÚDEZ, P.; BOLUDA, R. Effects of *Rosmarinus officinalis* and *Salvia officinalis* essential oils on *Tetranychus urticae* Koch (Acari: Tetranychidae). *Industrial Crops and Products*, New York, v. 48, p. 106-110, 2013.

LAGO, J. H. G.; MOREIRA, I. C.; TANIZAKI, T. M.; MORENO, P. R. H.; ROQUE, N. F.; LIMBERGER, R. P.; APEL, M. A.; HENRIQUES, A. T. Mono and sesquiterpenes from the leaf essential oil of *Xylopia brasiliensis* Spreng. (Annonaceae). *Journal of Essential Oil Research*, Carol Stream, v. 15, n. 6, p. 406-407, 2003.

LAGO, J. H. G.; REIS, A. A.; MARTINS, D.; CRUZ, F. G.; ROQUE, N. F. Composition of the leaf oil of *Xylopia emarginata* Mart. (Annonaceae). *Journal of Essential Oil Research*, Carol Stream, v. 17, n. 6, p. 622-623, 2005.

LEEUWEN, T. V.; VONTAS, J.; TSAGKARAKOU, A.; DERMAUW, W.; TIRRY, L. Acaricide resistance mechanisms in the two-spotted spider mite *Tetranychus urticae* and other important Acari: A review. *Insect Biochemistry and Molecular Biology*, Oxford, v. 40, n. 8, p. 563-572, 2010.

LIN, C. Y.; WU, D. C.; YU, J. Z.; CHEN, B. H.; WANG, C. L.; KO, W. H. Control of silverleaf whitefly, cotton aphid and kanzawa spider mite with oil and extracts from seeds of sugar apple. *Neotropical Entomology*, Londrina, v. 38, n. 4, p. 531-536, 2009.

LÜMMEN, P. Complex I inhibitors as insecticides and acaricides. *Biochimica et Biophysica Acta - Bioenergetics*, Amsterdam, v. 1364, n. 2, p. 287-296, 1998.

MADHUMITHA, G.; RAJAKUMAR, G.; ROOPAN, S. M.; RAHUMAN, A. A.; PRIYA, K. M.; SARAL, A. M.; KHAN, F. R. N.; KHANNA, V. G.; VELAYUTHAM, K.; JAYASEELAN, C.; KAMARAJ, C.; ELANGO, G. Acaricidal, insecticidal, and larvicidal efficacy of fruit peel aqueous extract of *Annona squamosa* and its compounds against blood-feeding parasites. *Parasitology Research*, Berlin, v. 111, n. 5, p. 2189-2199, 2012.

MARCIC, D. Acaricides in modern management of plant-feeding mites. *Journal of Pest Science*, New York, v. 85, n. 4, p. 395-408, 2012.

MCLAUGHLIN, J. L. Paw paw and cancer: Annonaceous acetogenins from discovery to commercial products. *Journal of Natural Products*, Cincinnati, v. 71, n. 7, p. 1311-1321, 2008.

MOREIRA, I. C.; ROQUE, N. F.; VILEGAS, W.; ZALEWSKI, C. A.; LAGO, J. H. G.; FUNASAKI, M. Genus *Xylopia* (Annonaceae): Chemical and biological aspects. *Chemistry & Biodiversity*, Weinheim, v. 10, n. 11, p. 1921-1943, 2013.

NAGASHIMA, F.; MURAKAMI, Y.; ASAKAWA, Y. Aromatic compounds from the Ecuadorian liverwort *Marchesinia brachiata*: a revision. *Phytochemistry,* New York, v. 51, n. 8, p. 1101-l104, 1999.

NASCIMENTO, F. D. C. do; DIAMANTINO BOAVENTURA, M. A.; ASSUNÇÃO, A. C. S.; SANTOS PIMENTA, L. P. Acetogeninas de anonáceas isoladas de folhas de *Rollinia laurifolia*. *Química Nova*, São Paulo, v. 26, n. 3, p. 319-322, 2003.

NEVES, I. A.; DA CAMARA, C. A. G. Acaricidal activity against *Tetranychus urticae* and essential oil composition of four croton species from caatinga biome in northeastern Brazil. *Natural Product Communications*, Ohio, v. 6, n. 6, p. 893-899, 2011.

NGADJUI, B. T.; LONTSI, D.; AYAFOR, J. F.; SONDENGAM, B. L. Pachypophyllin and pachypostaudins A and B: three bisnorlignans from *Pachypodanthium staudtii*. *Phytochemistry: Chemistry, Biochemistry, Molecular Biology*, New York, v. 28, n. 1, p. 231-234, 1989.

OLIVEIRA-FILHO, A. T. *TreeAtlan 2. 0, flora arbórea da América do Sul cisandina tropical e subtropical:* um banco de dados envolvendo biogeografia, diversidade e conservação. Belo Horizonte: Universidade Federal de Minas Gerais, 2010. Disponível em: <http://www.icb.ufmg.br/treeatlan/>. Acesso em: 20 nov. 2013.

OPREAN, R.; TAMAS, M.; ROMAN, L. Comparison of GC-MS and TLC techniques for asarone isomers determination. *Journal of Pharmaceutical and Biomedical Analysis*, Londres, v. 18, n. 1-2, p. 227-234, 1998.

PIMENTA, L. P. S.; NASCIMENTO, F. C.; BOAVENTURA, M. A. D. Acetogenins from the leaves of *Rollinia laurifolia*. *Helvetica Chimica Acta*, Basel, v. 88, n. 12, p. 3225-3231, 2005.

PONTES, W. J. T.; OLIVEIRA, J. C. S.; CÂMARA, C. A. G.; GONDIM JÚNIOR, M. G. C.; OLIVEIRA, J. V.; SCHWARTZ, M. O. E. Atividade acaricida dos óleos essencias de folhas e frutos de *Xylopia sericea* sobre o ácaro rajado (*Tetranychus urticae* Koch). *Química Nova*, São Paulo, v. 30, n. 4, p. 838-841, 2007.

POTENZA, M. R.; GOMES, R. C. O.; JOCYS, T.; TAKEMATSU, A. P.; RAMOS, A. C. O. Avaliação de produtos naturais para o controle do ácaro rajado *Tetranychus urticae* (Koch, 1836) (Acari:Tetranychidae) em casa de vegetação. *Arquivos do Instituto Biológico*, São Paulo, v. 73, n. 4, p. 455-459, 2006.

R DEVELOPMENT CORE TEAM. R: a language and environment for statistical computing. Vienna: Editora, 2013. Available at: <http://www.R-project.org>. Accessed at: 1 jul. 2013.

SAITO, M. L; ALVARENGA, M. A. Composição química de *Annona cacans* Warming. *Lecta-USF*, Bragança Paulista, v. 12, n. 1, p. 135-140, 1994.

SATO, M. E.; SILVA, M. Z.; RAGA, A.; SOUZA FILHO, M. F. Abamectin resistance in *Tetranychus urticae* Koch (Acari: Tetranychidae): selection, cross-resistance and stability of resistance. *Neotropical Entomology*, Londrina, v. 34, n. 6, p. 991-998, 2005.

SILVA, D. B. da; TULLI, E. C. O.; GARCEZ, W. S.; NASCIMENTO, E. A.; DE SIQUEIRA, J. M. Chemical constituents of the underground stem bark of *Duguetia furfuracea* (Annonaceae). *Journal of the Brazilian Chemical Society*, São Paulo, v. 18, n. 8, p. 1560-1565, 2007.

SILVA, E. L. M. da; ROBLOT, F.; MAHUTEAU, J.; CAVÉ, A. Coriadienin, the first annonaceous acetogenin with two double bonds isolated from *Annona coriaceae*. *Journal of Natural Products*, Cincinnati, v. 59, n. 5, p. 528-530, 1996.

SILVA, L. J. da. *R package*: DUNCAN test, Tukey test and Scott-Knott test. [S.l.: s.n.], 2010. Available at: <http://cran.r-project.org/web/packages/laercio/laercio.pdf>. Accessed at: 10 dez. 2013.

SOKOLYANSKAYA, M. P. The problem of the insect and mite resistance to insectoacaricides; resistance to organochlorines and organophosphates. *Resistant Pest Management Newsletter*, East Lensing, v. 19, n. 2, p. 62-66, 2010.

SOMKUWAR, D. O.; KAMBLE, V. A. Phytochemical screening of ethanolic extracts of stem, leaves, flower and seed kernel of *Mangifera indica* L. *International Journal of Pharma and Bio Sciences*, New Delhi, v. 4, n. 2, p. 383-389, 2013.

SONG, H. Y.; YANG, J. Y.; SUH, J. W.; LEE, H. S. Acaricidal activities of apiol and its derivatives from petroselinum sativum seeds against *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, and *Tyrophagus putrescentiae*. *Journal of Agricultural and Food Chemistry*, Easton, v. 59, n. 14, p. 7759-7764, 2011.

SOUSA, O. V.; DEL-VECHIO-VIEIRA, G.; ALVES, M. S.; ARAÚJO, A. A. L.; PINTO, M. A. O.; AMARAL, M. P. H.; RODARTE, M. P.; KAPLAN, M. A. C. Chemical composition and biological activities of the essential oils from *Duguetia lanceolata* St. Hil. Barks. *Molecules*, Switzerland, v. 17, n. 9, p. 11056-11066, 2012.

TEMPONE, A. G.; TREIGER BORBOREMA, S. E.; ANDRADE JÚNIOR, H. F. de; AMORIM GUALDA, N. C. de; YOGI, Á.; SALERNO CARVALHO, C.; BACHIEGA, D.; LUPO, F. N.; BONOTTO, S. V.; FISCHER, D. C. H. Antiprotozoal activity of Brazilian plant extracts from isoquinoline alkaloid-producing families. *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology,* Jena, v. 12, n. 5, p. 382-390, 2005.

UDDIN, M. R.; LI, X.; KIM, Y. B.; CHAE, S. C.; KIM, S. -J.; PARK, S. U. Phenolic compounds in different organs of tartary buckwheat (*Fagopyrum tataricum* Gaertn.) cultivars. *Australian Journal of Crop Science*, Lismore, v. 7, n. 12, p. 1861-1865, 2013.

VALENTE, P. P.; AMORIM, J. M.; CASTILHO, R. O.; LEITE, R. C.; RIBEIRO, M. F. B. In vitro acaricidal efficacy of plant extracts from Brazilian flora and isolated substances against *Rhipicephalus microplus* (Acari: Ixodidae). *Parasitology Research*, Berlin, v. 113, n. 1, p. 417-423, 2014.

VERONEZ, B.; SATO, M. E.; NICASTRO, R. L. Toxicidade de compostos sintéticos e naturais sobre *Tetranychus urticae* e o predador *Phytoseiulus macropilis*. *Pesquisa Agropecuária Brasileira*, Brasília, v. 47, n. 4, p. 511-518, 2012.

WANG, Z. W.; MA, W. W.; MCLAUGHLIN, J. L.; GUPTA, M. P. 2,4,5- trimethoxystyrene, a bioactive component of the bark of *Duguetia panamensis*. *Journal of Natural Products*, Cincinnati, v. 51, n. 2, p. 382-384, 1988.

YANAR, D.; KADIOĞLU, D.; GÖKÇE, A. Ovicidal activity of different plant extracts on twospotted spider mite (*Tetranychus urticae* Koch) (Acari: Tetranychidae). *Scientific Research and Essays*, Lagos, v. 6, n. 14, p. 3041-3044, 2011.

YAPI, T. A.; BOTI, J. B.; FÉLIX, T. Z.; AHIBO, A. C.; TOMI, F.; BIGHELLI, A. *Pachypodanthium staudtii* Engl & Diels from côte d'ivoire: composition of leaf, stem bark and roots oils. *European Journal of Scientific Research*, Victoria, v. 69, n. 1, p. 137-142, 2012.

ZHANG, Q.; DING, L.; LI, M.; CUI, W.; DING, W.; LUO, J.; ZHANG, Y. Action modes of *Aloe vera* L. extracts against *Tetranychus cinnabarinus* Boisduval (Acarina: Tetranychidae). *Agricultural Sciences*, Mississippi, v. 4, n. 3, p. 117-122, 2013.

ZUO, H. L.; YANG, F. Q.; ZHANG, X. M.; XIA, Z. N. Separation of *cis* and *trans*-asarone from *Acorus tatarinowii* by preparative gas chromatography. *Journal of Analytical Methods in Chemistry*, New York, v. 2012, p. 1-5, 2, Art. ID 402081, 2012.