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Effect of *Annona muricata* L. (1753) (Annonaceae) seeds extracts on *Tetranychus urticae* (Koch, 1836) (Acari: Tetranychidae)

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The use of botanical acaricides extracted from plants as an alternative to replace the chemical acaricides is an interesting and efficient option to control pests and ameliorate their toxic effects to humans and the environment. The aim of this work was to evaluate the effect of seed extracts of *Annona muricata* (Annonaceae) to control the mite *Tetranychus urticae* (Acari: Tetranychidae) using disks of 5.0 cm in diameter jack bean leaves, *Canavalia ensiformis* (Fabaceae) as a substrate. The ethanolic extract of the seeds showed the highest toxicity to the mite, with LC_{50} around 1.77 mg/ml, followed by hexanic and aqueous extracts, with LC_{50} estimated at 3.29 and 151.74 mg/ml, respectively. Abamectin caused mortality of 40% to *T. urticae* in a commercial dosage of 100 ml/100 L. The repellent effect of the ethanolic extract, the toxicity on eggs and the residual effect on mites were also evaluated. The concentrations of 0.61, 0.88 and 1.77 mg/ml, as well as Abamectin had neutral effects on *T. urticae* and the concentrations of 3.10, 5.11 and 12.07 mg/ml were repellent. The viability of the eggs when sprayed with the ethanolic extract (LC_{99}), Abamectin and the control was 9.5, 76.5 and 91.5%, respectively. The residual effect of ethanolic extract was 120 h after application (HAA), with mortality rates above 80%; Abamectin presented residual effect of 48 HAA with 33.3% mortality. In this way, the ethanolic extract of *A. muricata* proved to be a promising product to the control of *T. urticae*.

Key words: Botanical acaricide, spotted spider mite, soursop.

INTRODUCTION

The spider mite, *Tetranychus urticae* (Koch, 1836) (Acari: Tetranychidae), is one of the most important pests worldwide causing considerable losses in several economically important crops such as cotton, apple, vine, bean, strawberry, papaya, potatoes, tomatoes and other vegetables, ornamental and medicinal plants (Miresmailli and Isman, 2006; Moraes and Flechtmann, 2008).

Its importance as a pest is related to its high ability to

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develop resistance to chemical acaricides, its polyphagous habit, high breeding potential and short biological cycle. High infestations can cause discoloration of leaves, loss of photosynthetic capacity and, eventually, death of plants (Devine et al., 2001).

Chemical control with acaricides is still the most widely used method, but in many cases inefficiently, as its intensive use has selected resistant populations of mites (Sato et al., 2007), in addition, can cause upwelling of pests due to the mortality of natural enemies (Ferla and Moraes, 2006; Marsaro Júnior et al., 2012).

These negative effects of chemical acaricides could be mitigated by the use of botanical product extracted from plants with acaricide power. Their peculiar characteristics relating to efficiency, rapid degradation and low impact on natural enemies, humans and the environment may bring considerable advantages on their commercial use (Brito et al., 2008), as can be found in *Annona muricata* (soursop) seeds.

Forty-two species of Annonaceae family, distributed in 14 genera with emphasis on *A. muricata* and *Annona squamosa* L., with potential for insecticide/acaricide have already been reported (Oliveira and Pereira, 2009; Krinski et al., 2014).

The acetogenins present in these plants are important secondary metabolites that are responsible to the bioactivity of several species of Annonaceae (Alali et al., 1999), being found in the leaves, twigs, roots and seeds (Castillo-Sanchez et al., 2010). The acetogenins act as inhibiting mitocondrial electron transport and so affecting the NADH-ubiquinone oxidoreductase action (Álvarez et al., 2007).

The present work had the objectives to evaluate the lethal toxicity, repellent effect, egg toxicity and residual effect of seed extracts of *A. muricata* to control the spider mite *T. urticae*.

MATERIALS AND METHODS

The experiments were carried out at the Laboratory of Entomology and in the greenhouse of the Centrode Ciências Agrárias of the Universidade Federal de Alagoas (UFAL) in Rio Largo, Brazil.

Collecting and rearing spotted spider mite *T. urticae*

The mite was collected on infested rose bouquets from flower shops in Maceio, Alagoas. For identification and confirmation of species *T. urticae*, mounting was accomplished of specimen in lamina for microscopic and mayo of Hoyer. The identification was facilitated from the Entomology and Acarology Laboratory of UFAL *Campus* of Arapiraca. The mites were reared on plants of jack bean, (*Canavalia ensifomis* L. DC) (Fabaceae) inside cages of 0.50 × 0.50 × 0.50 m covered by fine mesh fabric, under room temperature of 26 ± 2°C, relative humidity 60 ± 10% and photophase of 12 h. This procedure was done to obtain pest population under the laboratory.

Obtaining seeds and preparation of the extracts

A. muricata seeds were washed in tap water and placed on paper towels to remove excess of water. The seeds were then placed in paper bags and dried out in an oven with air circulation at the temperature of 50°C for seven days. After that the seeds were grounded on a knife mill type Wiley to obtain a fine powder.

The extracts were prepared in the Laboratory of Natural Resources at UFAL. For the preparation of organic extracts, 6 kg of seeds powder was used, submitted to cold extraction in stainless steel percolator tube, first with hexane CH_3 (CH_2) $4CH_3$ (EH) by 24 h, then on the resulting pie was added ethanol (CH_3CH_2OH) (EE) for three times during 72 h. The solutions were filtered and concentrated in a rotary evaporator at 50ºC at reduced pressure. The aqueous extract H_2O (EA), was obtained from the seeds powder submitted to extraction in water at 35% (m / v) in stainless steel percolator tube for 48 h.

Toxicity of extracts

Pretesting were undertaken using different concentrations of the extracts to determine the upper limit values (100% mortality) and the lower limit (no mortality). The extracts were diluted in distilled water with Tween 80 (0.05%), at the following concentrations: for the aqueous extract (EA) 111.11; 176.47; 250.0; 333.33; 428.0; 538.46 mg/ml; for the hexanic extract (EH) 1.69; 3.39; 5.10; 6.81; 8.53; mg/ml 10.26 and for the ethanolic extract (EE): 0.88; 1.76; 3.53; 5.31; 7.10; 8.88 mg/ml. A control solution of distilled water and Tween 80 (0.05%) was used. The extracts were also compared with the application of the acaricide Abamectin as a positive control (Abamectin Nortox® EC 18 gl; Zhejiang Hisun Pharmaceutical Company Ltd) in a commercial dosage (100 ml/100 L).

Foliar disks of 5.0 cm in diameter of jack bean leaves were sprayed with the different treatments using a Potter Tower (Burkard, Rickmansworth, UK). The procedure was performed at a pressure of 5 psi/pol² in a volume of 2.3 ml extract, corresponding to a deposit of 1.9 ± 0.37 mg/cm², in accordance with the recommendation of the IOBC/WPRS (Reis et al., 1998).

The disks were then placed to dry out on paper towels at room temperature for an hour and put to float on water, inside Petri dishes (8.5 cm diameter), as the methodology described by Reis and Alves (1997). A total of 10 females of *T. urticae* were transferred to each disc, with the aid of thin brush bristles, totaling six replicates per treatment.

The mortality was evaluated 72 h after application (Sato et al., 2002) and lethal concentrations (LCs) estimated by the Probit analysis in the statistical program SAS (SAS Institute, 2002).

Repellent effect

For the repellency test EE and Abamectin was used. Disks (5.0 cm in diameter) were immersed for five seconds at each treatment or control (Tween 80 distilled water), placed side by side, and connected by a glass cover slip (18 × 18 mm). This set was placed on filter paper saturated with distilled water inside Petri dishes (14 cm in diameter), according to methodology adapted from Esteves Filho et al. (2010).

The tested concentrations of the EE were obtained on concentration-response curve, equivalent as LCs 10, 20, 50, 75, 90 and 99 and the Abamectin (100 ml/100 L). In the center of the cover slip 10 females of *T. urticae* were released, and after 2 h assessed the number of females in each disk, being 15 repetitions per treatment.

The repellency index (RI) was calculated by the formula: RI=2G/(G+P) according to Kogan and Goeden (1970), where G is

Table 1. Lethal concentration (LC) of the seed extracts of *Annona muricata* on females of *Tetranychus urticae*

Treatment	n[a]	DF[b]	Inclination ± SE	LC_{50} (mg/ml) (CI 95%)	LC_{99} (mg/ml) (CI 95%)	χ^{2c}	P
Aqueous extract	360	4	2.94 ± 0.35	151.74 (126.36-173.65)	933.38 (698.71-1472.0)	7.6	0.10
Hexanic extract	360	4	2.36 ± 0.43	3.29 (1.91-4.45)	31.87 (16.01-236.78)	9.2	0.06
Ethanolic extract	360	4	2.80 ± 0.26	1.77 (1.50-2.05)	12.07 (9.22-17.57)	7.7	0.10

[a]Number of mites used in each experiment; [b]Degree of freedom of Chi-square; [c]Chi-square.

Table 2. Repellent effect of the ethanolic extract (EE) of *Annona muricata* seeds on females of *Tetranychus urticae*.

Treatment	Concentration	Mean of repellency index[a] (±SD[b])	Effect
Abamectin (ml/L)	100 /100	1.18 ± 0.40	Neutral
Ethanolic extract (mg/ml)	0.61 (LC_{10})	0.74 ± 0.27	Neutral
	0.88 (LC_{20})	0.74 ± 0.27	Neutral
	1.77 (LC_{50})	0.86 ± 0.38	Neutral
	3.10 (LC_{75})	0.41 ± 0.19	Repellent
	5.11 (LC_{90})	0.32 ± 0.38	Repellent
	12.07 (LC_{99})	0.16 ± 0.22	Repellent

[a]Repellency index calculated according to Kogan and Goeden (1970). [b]SD: Standard deviation.

the number of mites in the treatment and P is the number of mites in control. The used safety interval period used to consider whether or not the treatment is repellent was obtained from the average of the calculated RI and its standard deviation (SD). If the average of RI was less than 1 - SD, the extract was repellent. If the average of RI was greater than 1 + SD, the extract was attractive; and if the average was between 1- SD and 1 + SD the extract was considered as neutral.

Toxicity on eggs

Females of *T. urticae* were transferred to disks (3.0 cm in diameter) inside Petri dishes (14.0 cm in diameter) containing moistened cotton. After 24 h, 10 eggs per disc were sprayed with the EE (LC_{99}), Abamectin (100 ml/100L) and Tween 80 distilled water (control), in Potter tower.

Twenty replicates per treatment were used. The evaluation of hatching of larvae was checked daily until 144 h after installation of the experiment. The data were subjected to variance analysis and means compared by Tukey test, at 5% probability, using the 7.7 Assistat beta version (Silva and Azevedo, 2009).

Residual effect

Jack bean plants of 12 days-old grown in a greenhouse (29 ± 1°C and 60 ± 5 to R.H.) were sprayed with a volume of 26 ml/plant of either ethanolic extract (LC_{99}), Abamectin (100 ml/100 L) or Tween 80 distilled water (10 plants per treatment). After periods of 0, 24, 48, 72, 96 and 120 h after application (HAA), leaf discs (5.0 cm in diameter), were collected and in laboratory, exposed to 10 adult females of *T. urticae.*

The mortality was assessed daily until 120 h after confinement (Schlesener et al., 2013). The data were subjected to variance analysis and means compared by Tukey test at 5% probability, in a factorial arrangement (3 × 6), using Assistat 7.7 beta (Silva and Azevedo, 2014).

RESULTS

The EE of *A. muricata* seeds have shown the highest toxicity to *T. urticae*, as it required lower concentration (1.78 mg/ml) to cause 50% mortality, followed by EH and EA, with LC_{50} estimated in 3.29 mg/ml and 151.74 mg/ml, respectively. The values of LC_{99} were 933.38 mg/ml to EA, 31.87 mg/ml to EH and 12.07 mg/ml to EE (Table 1).

The Abamectin treatment caused 40% mortality in a commercial dosage of 100 ml/100 L. Since the EE proved to be the most efficient in mortality of *T. urticae*, it was selected to evaluate its effect as repellent, toxicity on eggs and its residual effect.

The lowest concentrations tested LC_{10}, LC_{20} and LC_{50}, and the Abamectin presented neutral effect on *T. urticae*, not being efficient in repellency spider mite (Table 2). By contrast, the concentrations equivalent to LC_{75}, LC_{90} and LC_{99}, respectively, presented repellent effect on *T. urticae* (Table 2).

The viability of the eggs of *T. urticae* was affected by the application of EE (LC_{99}). Only 9.5 ± 2.8% of the eggs were viable in contact with the LC_{99} dosage. Significant differences was observes among the used extracts ($F = 139.2$; $P < 0.001$). The Abamectin presented 76.5 ± 5.25% of viable eggs and the control 91.5 ± 2.43% (Figure 1).

Significant differences were observed on the mortality of *T. urticae* among the used treatments in the following h after application (HAA) ($F = 3.57$; $P < 0.001$), with the

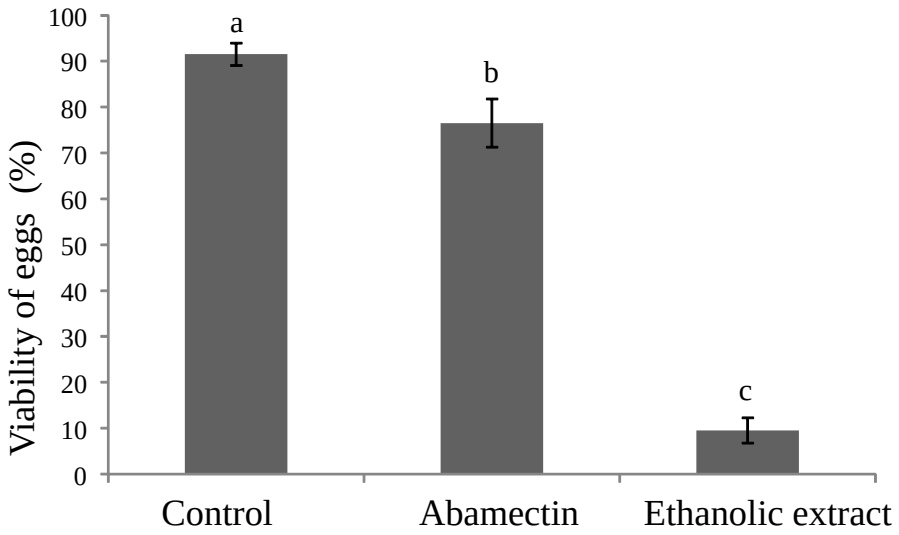

Figure 1. Viability of eggs of *T. urticae* exposed to LC_{99} of the ethanolic extract of *Annona muricata* seeds and Abamectin (100 ml/100 L).

exception of the period up to 72 HAA, showing no significant difference between the Abamectin and the control (Table 3).

In the period 0 HAA, it was observed that mortality of *T. urticae* was 93.3; 47.3 and 10.7% for EE, Abamectin and control, respectively. The EE had residual effect until the end of the experiment, 120 HAA with mortality above 80%. Abamectin afforded mortality rate of 12.7% 72 HAA (Table 3).

DISCUSSION

According to the obtained results, it was found that the ethanolic extract of *A. muricata* caused higher mortality for both LC (50 and 99), followed by hexanic extract mortality values. However, it is observed that the water does not work as efficient extractor for active ingredients of the plant in question.

The difference in the activity of aqueous and organic extracts (hexanic and ethanolic) is associated with the type of solvent (Trindade et al., 2000). Generally, the aqueous extract requires higher concentrations to cause pest mortality.

The acaricide effect of *A. muricata* extract is due to the presence of acetogenins, substances when used against arthropods, acted upon inhibiting the mitochondrial electron transport, affecting the action of NADH-ubiquinone oxidoreductase, which ultimately caused the death of these bodies (Álvarez et al., 2007).

Higher concentrations of EH were required to cause mortality of 50% of the *T. urticae* population compared to EE. These results seem to be related to the polarity of the solvents. Generally, non-polar solvents, such as hexanic (polarity 0.1) are less efficient than those with intermediate polarity, like ethanolic (polarity 4.3) (Potenza et al., 2005).

Other extracts and essential oils of botanical species were also efficient in controlling *T. urticae* (Ismail et al., 2011; Roh et al., 2011; Schlesener et al., 2013), these studies have shown that natural acaricides can be a good alternative to replace or reduce the use of chemical pesticides and, thus, mitigate the negative impacts that they cause.

Beyond the lethal toxic effect, other parameters have also been observed in this study showing the effects as repellent and ovicide. The results allow to understand that, even at concentrations below that able to cause 99% mortality in mite population, (LC_{99}); the extracts may cause repellence in the populations that may have survived its toxic effect. Essential oils of other botanical species, *Mentha longifolia* and *Salvia officialis* (Lamiaceae) and *Myrtus communis* (Myrtaceae) in addition to their lethal effect, they were also repellents to *T. urticae* with RC_{50} values estimated at 147.47; 138.80 and 164.41 µl/L, respectively (Motazedian et al., 2012).

The most significant benefit of using the ethanolic extract of *A. muricata* to control spider mite was the ovicide effect. In this case, it is observed that lethal effect was greater than 80%, this effect can be explained by the presence of acetogenins, substances with insecticidal activity acaricide, which are found in greater amounts in the seeds of *A. muricata* (Álvarez et al., 2007).

Natural herbal products based on azadirachtin and rotenone also caused egg infeasibility, evidencing that botanical acaricides are efficient in the control of eggs hatch of *T. urticae* (Brito et al., 2006; Duso et al., 2008). The ovicidal effect of an acaricide is a relevant property, because by controlling the initial stages of development of the mites, it may reduce or end the outbreak of

Table 3. Mortality of *T. urticae* is up to 120 h after application (HAA) ethanolic extract of *Annona muricata* seeds and Abamectin (100 ml/100 L).

Parameter	Mortality ± SE					
	0 HAA*	24 HAA	48 HAA	72 HAA	96 HAA	120 HAA
Control	10.7 ± 2.3^{cA}	9.0 ± 1.8^{cA}	10.7 ± 2.3^{cA}	6.0 ± 1.3^{bA}	8.0 ± 2.2^{cA}	4.7 ± 1.6^{cA}
Abamectin	47.3 ± 5.3^{bA}	32.0 ± 3.3^{bAB}	33.3 ± 4.9^{bAB}	12.7 ± 3.0^{bC}	23.3 ± 3.3^{bBC}	24.6 ± 5.8^{bBC}
Ethanolic extract	93.3 ± 3.3^{aA}	83.3 ± 6.9^{aA}	80.0 ± 4.8^{aA}	94.0 ± 4.1^{aA}	85.3 ± 6.1^{aA}	92.0 ± 4.3^{aA}
CV (%)				37.66		

Means followed by the same lower case letters in columns and capital letters on lines do not differ by Tukey test (P ≤ 0.05). *HAA: Hours after application; CV: coefficient of variation.

larvae and reduces the injuries caused by the pest (Esteves Filho et al., 2008).

The ethanolic extract of *A. muricata* seeds caused mortality during the 120 HAA, that is, throughout the experiment. Studies carried out with extracts of *Dieffenbachia brasiliensis* Veitch. (Areceae), *Ruta graveolens* L. (Rutaceae), *Allium cepa* L. (Liliaceae), *Agave angustifolia* Haw (Amaryllidaceae) and *A. squamosa* L. (Annonaceae) also reduced the population of *T. urticae* in 86.87, 83.95, 80.97, 76.30 and 75.40%, respectively, in plants of *Phaseolus vulgaris* L., within seven days after application (Potenza et al., 2006), similarly to the results obtained in this study.

The ethanolic extract of *A. muricata* proved to be a promising way to control *T. urticae*, showing satisfactory results in mite mortality, toxicity on eggs, repellency and residual effect.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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REFERENCES

Alali FQ, Liu XX, McLaughlin JL (1999). *Annona ceousacetogenins*: Recent progress. J. Nat. Prod. 62(2):504-540.

Álvarez CO, Neske A, Popish S, Bardón A (2007). Toxic effects of *Annona ceousacetogenins* from *Annona cherimolia* (Magnoliales: Annonaceae) on *Spodoptera frugiperda* (Lepidoptera: Noctuidae). J. Pest. Sci. 80:63-67.

Brito CH, Lopes EB, Albuquerque IC, Batista JL (2008). Avaliação de produtos alternativos e pesticidas no combate a cochonilha do-carmim na Paraíba. Rev. Biol. Ciênc. Terra 8:1-5.

Brito HM, Gondim Junior MGC, Oliveira JV, Câmara CAG (2006). Toxicidade de Natuneem sobre *Tetranychus urticae* Koch (Acari: Tetranychidae) e ácaros predadores da família Phytoseiidae. Ciênc. Agrotec. 30(4):685-691.

Castillo-Sánchez LHC, Jiménez-Osornio JJ, Delgado-Herrera MA (2010). Secondary metabolites of the Annonaceae, Solanaceae and Meliaceae families used as biological control of insects. Trop. Subtrop. Agroecosyst. 12(3):445-462.

Devine GJ, Barber M, Denholm I (2001). Incidence and inheritance of resistance to METI‐acaricidesin European strains of the two‐spotted spider mite (*Tetranychus urticae*) (Acari: Tetranychidae). Pest. Manag. Sci. 57(5):443-448.

Duso C, Malagnini V, Pozzebon A, Castagnoli M, Liguori M, Simoni S (2008). Comparative toxicity of botanical and reduced-risk insecticides to Mediterranean populations of *Tetranychus urticae* and *Phytoseiulus persimilis* (Acari Tetranychidae, Phytoseiidae). Biol. Control 47(1):16-21.

Esteves Filho AB, Oliveira JV, Gondim Jr MGC (2008). Toxicidade de acaricidas sobre diferentes estágios de vida de *Tetranychus urticae* Koch (Acari: Tetranychidae) em Mamoeiro. BioAssay 3(6):1-6.

Esteves Filho AB, Oliveira JV, Torres JB, Godim Jr MGC (2010). Biologia comparada e comportamento de *Tetranychus urticae* Koch (Acari: Tetranychidae) e *Phytoseiulus macropilis* (Banks) (Acari: Phytoseiidae) em Algodoeiro BollgardTM e Isolinha não-Transgênica. Neotrop. Entomol. 39(3):338-344.

Ferla NJ, Moraes GJ (2006). Seletividade de acaricidas e inseticidas a ácaros predadores (Acari: Phytoseiidae) encontrados em seringueira no centro-oeste do Brasil. Cienc. Rural 36(2):357-362.

Ismail MSM, Ghallab MMA, Soliman MFM, AboGhalia AH (2011). Acaricidal activities of some essential and fixed oils on the two spotted spider mite, *Tetranychus urticae*. Egypt. Acad. J. Biol. Sci. 3(1):41-48

Kogan M, Goeden RD (1970). The host-plant range of *Lema trilineata daturaphila* (Coleoptera: Chrysomelidae. Ann. Entomol. Soc. Am. 63(4):1175-1180.

Krinski D, Masaroli A, Machado M (2014). Potencial inseticida de plantas da família Annonaceae. Rev. Bras. Frutic. 36:225-242.

Marsaro Júnior AL, Sato ME, de Aguiar RM, Vieira GB, da Silva Júnior RJ, Mineiro JLC (2012). Efeito de acaricidas sobre *Schizotetranychus hindustanicus* (Hirst) (Acari: Tetranychidae) e ácaros predadores em citros no estado de Roraima, Brasil. Arq. Inst. Biol. 79(1):75-83.

Miresmailli S, Isman MB (2006). Efficacy and persistence of rosemary oil as an acaricide against two-spotted spider mite (Acari: Tetranychidae) on greenhouse tomato. J. Econ. Entomol. 99(6):2015-2023.

Moraes GM, Flechtamann CHW (2008). Manual de Acarologia: acarologia básica e ácaros de plantas cultivadas no Brasil. Holos, Ribeirão Preto, 288p.

Motazedian N, Ravan S, Bandani AR (2012). Toxicity and repellency effects of three essential oils against *Tetranych usurticae* Koch (Acari: Tetranychidae). J. Agric. Sci. Technol.14(2):275-284.

Oliveira AC, Pereira MJB (2009). Efeito antialimentar do

extrato metanólico de *Annona crassiflora* Mart. sobre o percevejo marrom *Euschistus heros* (Fabr. 1798) (Heteroptera: Pentatomidae). Rev. Bras. Agroecol. 4(2):2633-2636.

Potenza MR, Gomes RCO, Jocys T, Takematsu AP, Ramos ACO (2006). Avaliação de produtos naturais para o controle do ácarorajado *Tetranychus urticae* (Koch, 1836) (Acari: Tetranychidae) em casa de vegetação. Arq. Inst. Biol. 73:455-459.

Potenza MR, Takematsu AP, Jocys T, Felicio JDF, Rossi MH, Nakaoka Sakita M (2005). Avaliação acaricida de produtos naturais para o controle de ácaro vermelho do cafeeiro *Oligonychus ilicis* (McGregor) (Acari: Tetranychidae). Arq. Inst. Biol. 72(4):499-503.

Reis PR, Alves EB (1997). Criação do ácaro predador *Iphiseiodes zuluagai* Denmark Muma (Acari: Phytoseiidae) em laboratório. An. Soc. Entomol. Bras. 26:565-568.

Reis PR, ChiavegatoLG, Moraes GJ, Alves EB, Sousa EO (1998). Seletividade de agroquímicos ao ácaro predador *Iphiseiodes zuluagai* Denmark & Muma (Acari: Phytoseiidae). An. Soc. Entomol. Bras. 27:265-274.

Roh HS, Lim EG, Kim J, Park CG (2011). Acaricidal and oviposition deterring effects of santalol identified in sandalwood oil against two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae). J. Pest. Sci. 84(4):495-501.

SAS INSTITUTE (2002). User's guide: statistics version 9.1 – SAS Institute Inc. Cary/United States of America.

Sato ME, da Silva M, Gonçalves LR, de Souza Filho MF, Raga A (2002). Toxicidade diferencial de agroquímicos a *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) e *Tetranychus urticae* Koch (Acari: Tetranychidae) em morangueiro. Neotrop. Entomol. 31:449-456.

Sato ME, da Silva MZ, Cangani KG, Raga A (2007). Seleções para resistência e suscetibilidade, detecção e monitoramento da resistência de *Tetranychus urticae* ao acaricida Clorfenapir. Bragantia 66(1):89-95.

Schlesener DCH, Duarte AF, Guerrero MFC, da CunhaUS NDE (2013). Efeitos do nim sobre *Tetranychus urticae* Koch (Acari: Tetranychidae) e os predadores *Phytoseiulus macropilis* (Banks) e *Neoseiulus californicus* (McGregor) (Acari:Phytoseiidae). Rev. Bras. Frutic. 35(1):59-66.

Silva FAS, Azevedo CAV (2009). Principal Components Analysis in the Software AssistatStatistical Attendance. In: World Congresson Computers in Agriculture, 7, Reno-NV-USA: American Society of Agricultural and Biological Engineers pp. 393-396.

Trindade RCP, Marques IMR, Xavier HS, Oliveira JV (2000). Extrato metanólico da amêndoa da semente de nim e a mortalidade de ovos e lagartas da traça-do-tomateiro. Sci. Agric. 57(3):407-413.