Original Article

CONTROL OF SILVERLEAF WHITEFLY IN CASSAVA GROWN IN THE GREENHOUSE TREATED WITH *Anacardium humile* (Anacardiaceae) extract

CONTROLE DE MOSCA-BRANCA EM MANDIOCA CULTIVADA EM CASA DE VEGETAÇÃO TRATADA COM EXTRATO DE Anacardium humile *(Anacardiaceae)*

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ABSTRACT: *Bemisia tuberculata* (Bondar, 1923) is the most important pest of cassava crops. This work describes the effect of different concentrations of $CHCl_3$ extract from the leaves of *Anacardium humile* in relation to *Bemisia tuberculata*, under laboratory conditions, in cassava plants and chemical composition of this extract. Fractionation of the $CHCl_3$ extract yielded the triterpene acids: oleanolic, ursolic and betulinic. Their structures were determined on the basis of spectral data (NMR-1D and 2D) and subsequent comparisons with the literature. Anacardic acid was identified in $CHCl_3$ extract by high performance liquid chromatography (HPLC). $CHCl_3$ extract caused mortality of whitefly nymphs of *B. tuberculata* from 66.30 to 74.90%, in doses tested. The insecticidal activity of $CHCl_3$ extract of *A. humile* on the nymphs of the whitefly suggests that pentacyclic triterpenes and anacardic acid have potential insecticidal activity.

KEYWORDS: *Manihot esculenta. Bemisia tuberculata*; *Anacardium humile.* Botanical insecticide.

INTRODUCTION

The whitefly *Bemisia tuberculata* (Bondar 1923) (Hemiptera: Aleyrodidae) is one of the main problems for producing healthy cassava crops in the state of Mato Grosso do Sul (ANDRADE FILHO et al., 2010). This pest causes direct damage to the host plant, such as severe dehydration, curling of apical leaves, chlorosis, yellowing and necrosis. Indirect damage has also been reported, due to the possibility of transmitting the virus and the formation of sooty mold on the leaves, which jeopardizes photosynthesis. The pest may also transport phytophagous acari, as has been reported in cucumber plants in Venezuela (BAUTISTA et al., 2005).

Currently, control of whitefly in cassava plants is done using synthetic insecticides, mainly from the phosphorates, carbamates and pyrethroids, which are also recommended for crops which are attacked by other Aleyrodidae species (SCARPELLINI et al., 2002). However, many insect pests can develop a resistance mechanism to these chemical products, not to mention the latter's undesirable effects, especially contamination of the environment and residues left on food crops (VIEGAS, 2003).

As a result, viable strategies are being sought to reduce insect populations and at the same time decrease the problems frequently caused by synthetic products. The use of plant extracts with insecticidal activity has produced promising results in combating insects (BALDIN et al., 2007). These "phytoinsecticides", used in association with other control methods, are an option for self-sustainable agricultural systems (CAVALCANTE et al., 2006). They can also be explored as target molecules for the synthesis of new insecticides, which has advantages over synthetic ones, such as practicality, storage time and new action mechanisms in relation to the target insect, while being economically and ecologically viable (VIEGAS, 2003).

Among the plant extracts investigated for the control of whitefly, especially *Bemisia tabaci*, those belonging to species from the Meliaceae family (*Melia azedarach* L.; *Trichilia pallida* Swartz and *Azadirachta indica* A. Juss) have been particularly promising. These extracts and/or essential oils have presented significant results in mortality of nymphs and eggs (SOUZA et al., 2004). Other botanical species that have presented promising results in the control of whitefly (*B. tabaci*) are: aqueous extract and four fractions of (water, water/methanol, methanol, and diethyl ether) *Quassia amara* L. (FLORES et al., 2008); aqueous extracts of *Prosopis juliflora* (Sw.) DC., *Myracrodruon urundeuva* Fr. All., *Mimosa caesalpiniifolia* Benth (CAVALCANTE et al., 2006); aqueous extracts of *Leucaena leucocephala* (Lam.) de Wit, *Sterculia foetida* L.

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(VASCONCELOS et al., 2006) and aqueous extracts of *Azadirachta indica* A. Juss., *Trichilia pallida* Swartz, *Chenopodium ambrosioides* Linn., *Piper nigrum* L., *Melia azedarach* L., *Ruta graveolens* L., *Ricinus communis* L., *Mentha pulegium* L., *Tagetes erecta* L., *Eucalyptus citriodora* HK., *Cymbopogon nardus* L. and *Coriandrum sativum* L. (BALDINI et al., 2006).

For *Bemisia argentifolii*, a whitefly that attacks melon crops, azadirachtin, which is a compound isolated from neem, *Azadirachta indica* (Meliaceae), presented promising results in control of this insect in the greenhouse (SILVA et al., 2003). In a study carried out by Bleicher et al. (2007), the aqueous extract from neem seeds, as well as azadirachtin, was active in the control of whitefly nymphs.

With regard to the family Anacardiaceae, constituted by 76 genera and 600 species in Brazil, the genus *Anacardium* is the subject of a large number of investigations into insecticidal activity in the control of insects that attack agricultural crops and transmit diseases (VIEGAS, 2003; PORTO et al., 2008).

Anacardium humile St. Hill (Cerrado cashew/*cajuzinho-do-cerrado*), which is endemic to Brazil's Cerrado (savannah) biome, is used in folk medicine in various parts of the country, including the state of Mato Grosso do Sul (PORTO et al., 2008; LUIZ-FERREIRA et al., 2008). In terms of insecticidal potential, oil from the leaves causes considerable mortality among larvae of *Aedes aegypti* L., unlike the hexane, ethanol and aqueous extracts at various concentrations, which were considered inactive (PORTO et al., 2008). On the other hand, the aqueous extract (ANDRADE FILHO et al., 2010) and oil (ANDRADE FILHO et al., 2013) of cerrado-cashew leaves was active against *Bemisia tuberculata*.

Bearing in mind the damage that can be done by whitefly in cassava plantations, especially in Mato Grosso do Sul, and the harm that can be caused by indiscriminate use of chemical control, for the environment and consequently for humans, alternative methods of controlling this pest are being sought. For this, one of the proposals for the use of natural insecticides is the aqueous extract of *Anacardium humile* (ANDRADE FILHO et al., 2010).

The use of substances of plant origin in the form of isolates presents advantages over synthetic products, such as lower toxicity, better biodegradability and selective action toward the target organism (insect, microorganism or other) (VIEGAS, 2003). Furthermore, isolating substances that have been biologically active for decades allows their use as prototypes for the synthesis of new insecticides (PUPO; GALLO, 2007). This work was undertaken to evaluate the effect of different concentrations of $CHCl_3$ extract from the leaves of *Anacardium humile* in relation to *Bemisia tuberculata*, under laboratory conditions, in cassava plants and chemical composition of this extract.

MATERIAL AND METHODS

Experiment under greenhouse conditions

The experiment was carried out in a greenhouse in São Vicente Experimental Farm, in Campo Grande, Mato Grosso do Sul, during the period from December 2007 to September 2008. The greenhouse is composed of two rooms, both at a temperature of 26±4°C and relative humidity of 80 ± 10%. One room was used for the production of seedling and the watering system was automated for this room only, watering three times a day for 3 minutes, with temperature adjusted to 26±4°. In the other room of the greenhouse the stock collection of whitefly *B. tuberculata* was kept, as well as maintaining plants infested with one-day-old eggs, for observations of biological parameters.

Cassava cuttings

The *Manihot esculenta* Crantz (cultivar Paraná) plants were produced in black plastic sacks of two kilograms from stalk cuttings taken from an established crop in the experimental area. The plantlets were used for the experiments when they were close to 30 days of age. The soil used was classified as Dystrophic Red Latosol, and fertilization took place according to soil analysis, using 80 kg ha^{-1} of Yoorin thermophosphate and 2.0 t ha^{-1} of organic compounds based on pig manure.

Biological assays

The treatments were: $CHCl_3$ extract from *A. humile* leaves and the synthetic chemical product Thiametoxan, at concentrations of 2, 0.8, 0.4 and 0.006% (P/V), and the blank control where only water was sprayed.

For each treatment five cassava plants, at two months old, were used. Each plant received spray on two leaves, making 10 repetitions per treatment. Twenty individuals of whitefly *B. tuberculata*, taken from the breeding stock, were imprisoned within "Voil" cages adapted to the size of the leaves. After a period of 24 hours the cages were removed, so that the eggs in treatment were one day old.

Spraying was done 13 days after infestation, the moment at which most of the nymphs at first instar were already fixed to the leaves. The products were prepared immediately before application and applied with a manual sprayer until dripping point, on the underside of the leaves.

The evaluations, which were of mortality per treatment, were done when the pseudo-pupae appeared, at the intermediate phase between juvenile and adult, and involved daily monitoring.

Plant material

Leaves of *A. humile* were collected in a patch of cerrado vegetation (20º26'20.64''S; 54º32'26.78''W), at Campo Grande, Mato Grosso do Sul, Brazil, in July 2008. The plant material exsiccatae are stored in the Herbarium of Anhanguera University – Uniderp (RG. 6237), Campo Grande, Mato Grosso do Sul.

Isolation of the chemical constituents

The chloroform extract ($CHCl_3$), 3.2 g, was prepared from 500 g of leaves of *A. humile*, triturated and extracted successively with hexane, $CHCL_3$, ethylic alcohol (95%) and water in a Soxhlet extractor (4 hours), using the residue of the first extraction to return to the next extraction, according to the methodology described in the literature for the species under study (PORTO et al., 2008).

Part of the $CHCl_3$ extract (2.14 g) of *A. humile* was fractionated in a classic column (CC) using organic solvents of graded polarity as the mobile phase: hexane; hexane/dichloromethane (2:1 v:v, 1:1 v:v, 1:2 v:v), dichloromethane; dichloromethane /methanol (1:1 v:v) and methanol, and from this 19 recombinant fractions were obtained through chromatographic behavior (CCD) and yield. Fractions F_{15} (5.3 mg) and F_{16} (8.4 mg), after recrystallization in acetone/MeOH, provided a pure oleanane skeleton substance and a mixture of triterpene acids.

The NMR spectra (uni- and bi-dimensional) were obtained in a Varian spectrometer, model Mercury plus BB, operating at 300 MHz for ^{1}H and at 75.5 MHz for ^{13}C. The chemical displacements were given in ppm, with the internal reference being tetramethylsilane TMS (d = 0.0 ppm) or the solvent itself. The solvent used was $CDCl_3$. For the column chromatographs (CC) silica gel 60 was used (0.063-0.200 mm, Merck). Visualization of the compounds in CCD (chromatoplates of silica gel GF_{254} with aluminum support, 0.2 mm, Merck), was carried out by irradiation with ultraviolet light at 254 and 366 nm and/or by spraying with a solution of H_2SO_4/MeOH (1:1), H_2SO_4/anisaldehyde/acetic acid (1:0,5:50 mL), both followed by heating.

High performance liquid chromatography (CLAE)

The $CHCl_3$ extract was further submitted to High Performance Liquid Chromatography, model Varian 210, Diode Arrangement Detector (DAD) and software Star WS (workstation). The column used was reverse phase C-18 (25 cm x 4.6 mm x 5 μm) and the pre-column (2.5 cm x 3 mm) was of the same phase as the column (Phenomenex). The elution process was carried out with a graded program of solvent A: acetonitrile/water/acetic acid (66/33/2v:v:v) and B: with 100% of tetrahydrofurane. The pump flow rate was 1 mL/min and 20 μL was injected. The analysis was conducted at 22ºC.

Statistical analysis

ANOVA was used in the statistical treatment and means were compared by Tukey test ($p \leq 0.05$). The results were expressed as mean ± standard error of the mean.

RESULTS AND DISCUSSION

Study of the toxicity of the $CHCl_3$ extract on *B. tuberculata* nymphs

As expected, the Thiametoxan product demonstrated a shock effect, with total mortality still at nymph stage. The $CHCl_3$ extract, in its turn, produced mortality that was significantly different from that of the blank control, all tested concentrations, varied from 66.30 to 74.90%. But all the concentrations showed different behaviour from that of the control, where natural mortality was on average 18.01% (Table 1).

However, all the surviving nymphs died in the pseudo-pupa phase at all concentrations. So individuals that survived the juvenile phase died in the pseudo-pupa phase, showing the gradual increase in mortality.

In this study, considering the efficiency of the tested products and doses, it was noticed that the synthetic chemical product Thiametoxan shows 100% of control. However, the $CHCl_3$ extract of *A. humile* effectively reduced the population by between 75.95 and 72.83%, for doses between 2 and 0.006%, respectively (Table 2). In other words, the extract reduced the nymph population by more than 70%, but caused 100% reduction of the population, considering the adults that would be born from these pseudo-pupae.

Table 1. Effect of the $CHCl_3$ extract from leaves of *Anacardium humile* and of the synthetic chemical product (Thiametoxan) at various concentrations, on mortality (± standard error) of *Bemisia tuberculata* nymphs in the greenhouse, Temp.: 26±4°C. Campo Grande, MS.

Concentration	Nymphs	Pseudo-pupas	% Total mortality
2%	74.90 ± 5.85 a	100 ± 0.00 a	100 ± 0.00 a
0.8%	74.39 ± 14.35 a	100 ± 0.00 a	100 ± 0.00 a
0.4%	70.95 ± 8.35 a	100 ± 0.00 a	100 ± 0.00 a
0.006%	66.30 ± 10.07 a	100 ± 0.00 a	100 ± 0.00 a
Control	18.01 ± 11.16 b	14.81 ± 3.43 b	32.02 ± 4.64 b
Thiametoxan	100 ± 0.00 a	------	100 ± 0.00 a
C.V.%	5.37	XX	XX

** Means followed by the same letter in the same column, do not differ by Tukey test (P>0.05).

Synthetic chemical products have an immediate effect on insects, while the biocide effect of plant products is observed some days after application.

For the aqueous extract from leaves of *A. humile* at concentrations of 2.0; 0.8; 0.4 and 0.006% partial mortality was provoked in nymphs of *B. tuberculata*, but total mortality of “pseudo-pupae” at all tested concentrations and lengthening of the juvenile phase cycle at all the tested dosages (ANDRADE FILHO et al., 2010). The essential oil from the leaves of *A. humile* is a supplier of molecule(s) insecticide(s) and causes a deleterious effect on *B. tuberculata* with increased of the the juvenile phase, nymphal and total mortality of “pseudo-pupae” dosages (ANDRADE FILHO et al., 2013).

As well as mortality, plant products can provoke other effects on insects, such as prolonged juvenile phase, reduction in feeding, deformed individuals, reduction of egg-laying and fewer fertile eggs, observed in sublethal doses. Adverse effects occasioned by products based on neem *Azadirachta indica* (Meliaceae) were observed in sublethal dosages. As a consequence, with the application of plant products it can be observed that the population is reduced because of various deleterious effects, which brings about diminished infestation over time (MARTINEZ; VAN EMDEN, 1999).

In the Meliaceae family (*Melia azedarach*; *Trichilia pallida* and *Azadirachta indica*), the limonoids, are considered responsible for the control, principally the substance azadirachtin, of *Bemisia tabaci* Gennadius (CUBILO; HAJIA, 1999; BLEICHER et al., 2007).

Together with their toxic activity, natural biocides should present other properties, such as: efficiency even at low concentrations, absence of toxicity to other organisms, absence of phytotoxicity, ease of acquisition, economic viability and non-accumulation in animal fatty tissue (VIEGAS, 2003). In this work, phytotoxicity of the $CHCl_3$ extract was not observed in any of the tested concentrations, while the synthetic chemical product caused the death and fall of six leaves out of the 50 under treatment.

Composition of the extract

Fractionation of the $CHCl_3$ extract obtained from leaves of *Anacardium humile* (Anacardiaceae) resulted in the isolation of a pure substance, oleanolic acid, and a mixture of triterpene acids: oleanolic acid; ursolic acid and betulinic acid. Structural elucidation took place by means of uni- and bi-dimensional spectrum analysis of RMN ^{1}H and ^{13}C and by comparison with data described in the literature (TAKETA et al., 2004).

In the RMN ^{1}H spectrum signals between δ_H 0.78 and δ_H 1.12 can be observed, which refer to the seven methyl groups corresponding to the hydrogens of the cited skeletons. The signal at δ_H 5.33 (m) refers to olefinic hydrogen linked to the C-12. The signal at δ_H 3.43 (m) belongs to carbinolic hydrogen linked to the C-3. The signal at δ_H 2.79 (m; H-18) is characteristic of oleanolic acid, while for ursolic acid a displacement of about δ_H 2.15 (1H, dd, H-18) is expected. To confirm the presence of the oleanic nucleus, as well as analyzing the signals in the RMN ^{1}H spectrum, the correlations between olefinic hydrogen H-12 and the hydrogens H-18 and H-11 were analyzed in the COSY-^{1}H-^{1}H

experiment, the RMN ^{13}C spectrum data, HMQC and in comparisons with data from the literature (TAKETA et al., 2004).

For the mixture obtained, in the RMN ^{1}H spectrum the presence of duplicated signals was evident in the region of δ_H 5.41 and δ_H 3.61 with regard to oleanolic and ursolic acid, and additional signals were seen in the region of δ_H 4.78 to δ_H 4.55 with regard to betulinic acid. The presence of signals in triplicate was also seen in the RMN^{13}C spectrum, in agreement with the composition of the proposed mixture. The additional signals in δ_H 154.9 and 107.7 are specific to C_{29} and C_{20}, respectively, for betulinic acid. These data were compared with those described in the literature (WENKERT et al., 1978).

The anacardic acids, monoene (T_r=3.80 min) and diene (T_r=4.53 min), were identified in the $CHCl_3$ extract from leaves of A. humile by high performance liquid chromatography with diode array detector (Figure 1).

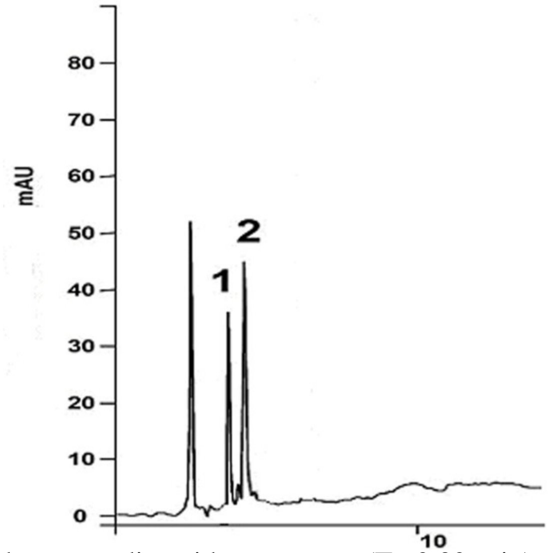

Figure 1. Chromatogram of the anacardic acids, monoene (T_r=3.80 min) and diene (T_r=4.53 min), by high performance liquid chromatography with diode array detector.

The pentacyclic triterpenes (ursolic acid, oleanolic acid and betulinic acid) isolated from the $CHCl_3$ extract constitute the biggest class of secondary products from plants, being mainly characterized as defense compounds against phytophages (MAIRESSE, 2005). Triterpenes, moreover, have the function of protecting plants, and their insecticidal action is apparently due to acetylcholinesterase inhibition in insects (COELHO et al., 2009).

Furthermore, depending on the class of triterpenes, these can also cause physiological disturbances, altering the development and functionality of various pest species, mainly due to inhibition or retardation of development and growth, disturbances in maturation, reduction in reproductive capacity and feeding repulsion (appetite suppression), which may lead to the death of predatory insects by inanition or direct toxicity (NDUMU; GEORGE, 1999; VIEGAS, 2003).

Among the pentacyclic triterpenes dealt with in this article, oleanolic acid and ursolic acid have an effect on the fluidity and stability of the liposomal membrane, as well as presenting strongly antifeedant action on other insects such as *Spodoptera litura* (HANS et al., 1997).

Oleanolic acid, specifically, is found in various plant sources, and its concentration depends on the seasonal temperature. It carries out important biological activities in the pharmacological area (YIN; CHAN, 2007) and, in terms of insecticidal activity, this acid and its derivatives are feeding deterrents for *Leptinotarsa decemlineata* (XU et al., 2009). It is also cited by Yin and Chan (2007) as being responsible for anti-ecdysis activity in *Rhodnius prolixus* (Reduviidae).

A significant number of biological activities have also been reported for ursolic acid (YIN; CHAN, 2007). This triterpene works as a formicide against caterpillars of *Achaea janata* (Noctuidae) (CHAMDRAMU et al., 2003). Insecticidal and repellent action has been confirmed for this substance, mainly from its derivatives (LUNZ et al., 2007). Ursolic acid isolated from leaves and stalks of *Duboisia myoporoides* (Solanaceae) presented deterrent action on feeding in *Spilosomu obliqua* and *S. litura* (Shukla et al. 1996).

In the case of betulinic acid, its action occurs via a steroidal mechanism, and this is thought to interfere in the metamorphosis and development of insects (SCHOWALTER, 2006).

Anacardic acids and derivatives have been reported in a number of works as having insecticidal action, particularly for Aedes sativum (CONSOLI et al., 1988) and for Aedes aegypti (LOMONACO et al., 2009).

The $CHCl_3$ extract caused an effect on the mortality of nymphs from 66.30 to 74.90% at all doses tested, as shown in table 1. The chloroform extract's insecticidal activity on whitefly nymphs (B. tuberculata) suggests that particularly the pentacyclic triterpenes and the phenolic lipids may be used as models for developing new insecticides to control this insect.

CONCLUSION

This work therefore confirms the importance of future investigations to test these triterpenes in isolation and/or evaluate their deleterious effect on nymphs of *B. tuberculata* and on other species of the genus. Specifically, this work identified the substances (pentacyclic triterpenes and anacardic acid) for the first time in this plant species.

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RESUMO: A *Bemisia tuberculata* (Bondar, 1923) é atualmente a principal praga da cultura da mandioca. Este trabalho descreve o efeito de diferentes concentrações do extrato $CHCl_3$ das folhas de *Anacardium humile*, em relação à *Bemisia tuberculata*, em condições de laboratório, em plantas de mandioca, além da composição química deste extrato. O fracionamento do extrato $CHCl_3$ forneceu os triterpenos ácidos: oleanólico, ursólico e betulínico. As estruturas foram determinadas com base nos dados espectrais (RMN-1D e 2D) e subsequente comparação com dados da literatura. O ácido anacárdico foi identificado no extrato $CHCl_3$ por cromatografia líquida de alta eficiência (CLAE). O extrato $CHCl_3$ causou efeito na mortalidade de ninfas de mosca branca *B. tuberculata* de 66,30 a 74,90% em todas as dosagens testadas (2; 0,8; 0,4 e 0,006%). A atividade inseticida do extrato $CHCl_3$ de *A. humile* sobre as ninfas de mosca-branca sugere que os triterpenos isolados podem ser utilizados como modelo para o desenvolvimento de novos inseticidas para o controle deste inseto.

PALAVRAS-CHAVE: *Manihot esculenta; Anacardium humile;* Inseticida botânico.

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