Ciência Rural, Santa Maria, v.47: 11, e20170092, 2017 http://dx.doi.org/10.1590/0103-8478cr20170092

ISSNe 1678-4596
CROP PROTECTION

Phytochemical characterization and bioactivity of ethanolic extracts on eggs of citrus blackfly

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ABSTRACT: *The main objectives of this study were to determine the content of secondary metabolites (carotenoids, flavonoids, phenolic compounds, and tannins) of Argemone mexicana L., Ipomoea carnea Jacq. subsp. Fistulosa (Martius ex Choisy), Amorimia rigida (A.Juss.) W. R. Anderson, Ricinus communis L. and Syzygium aromaticum (L.) Merr. & L. M. Perry using UV-VIS spectroscopy, and evaluating the bioactivity of the ethanolic extracts on citrus blackfly eggs (Aleurocanthus woglumi Ashby, 1915). Pera sweet orange leaves infested with citrus blackfly eggs were treated by immersion in 0.5, 1.0, 5.0, and 10%; each replicate consisted of 30 eggs. The experimental design was completely randomized, with seven treatments and four replications. Three immersions of leaves with eggs were performed, and the mortality was evaluated seven days after the procedure. Leaves were placed in Petri dishes and kept in incubators [25±1°C; relative humidity (RH) 60±5% and 12 hours]. S. aromaticum peduncle presented high content of tannins and phenolic compounds, while R. communis leaves showed high content of phenolic compounds. The commercial product Bioneem© caused egg infeasibility greater than 85% at all concentrations. Treatments (10%) that caused the greatest egg mortality were Bioneem© (94.74%), R. communis extract (81.58%), and the extract of S. aromaticum peduncle (80.57%).*
Key words: *Aleyrodidae, Citrus blackfly, Phytochemical bioprospecting, Ricinus communis, Syzygium aromaticum.*

Caracterização fitoquímica e bioatividade de extratos etanólicos em ovos de mosca-negra-dos-citros

RESUMO: *Os principais objetivos deste estudo foram determinar, utilizando espectroscopia UV-VIS, os compostos secundários (carotenoides, flavonoides, compostos fenólicos e taninos) das espécies Argenome mexicana L., Ipomoea carnea Jacq. subsp. Fistulosa (Martius ex Choisy), Amorimia rigida (A.Juss.) W. R. Anderson, Ricinus communis L. e Syzygium aromaticum L. (L.) Merr. & L. M. Perry, bem como avaliar a bioatividade de extratos etanólicos sobre ovos da mosca-negra-dos-citros (Aleurocanthus woglumi Ashby, 1915). Folhas de laranja Pêra infestadas com mosca-negra-dos-citros foram imersas nos tratamentos, a 0,5%, 1,0%, 5,0% e 10% de concentração; cada replicata foi constituída por 30 ovos. O delineamento foi inteiramente casualizado, sete tratamentos e quatro repetições. Foram realizadas três imersões das folhas com ovos, tendo sido avaliada a mortalidade sete dias após as imersões. As folhas foram alocadas em placa de Petri e mantidas em câmaras climáticas (25±1°C; U.R 60±5% e 12 horas). O pedúnculo do craveiro-da-india apresentou alto teor de taninos e compostos fenólicos, e folhas de mamona apresentavam alto teor de compostos fenólicos. O produto comercial Bioneem©, em todas as concentrações, causou inviabilidade de ovos superior a 85%. Os tratamentos (10%) que causaram a maior inviabilidade de ovos foram o Bioneem© (94,74%), o extrato de mamona (81,58%) e o extrato do pedúnculo do craveiro-da-india (80,57%).*
Palavra-chave: *Aleyrodidae, Aleurocanthus woglumi, Bioprospecção, Ricinus communis, Syzygium aromaticum.*

INTRODUCTION

Citrus blackfly (*Aleurocanthus woglumi* Ashby, 1915), an exotic pest widely disseminated in Brazil, is capable of infecting more than 300 species including fruit trees and ornamental plants. Citrus species, considered its primary hosts, are highly susceptible to infestation by this pest (EPPO, 2008; RAGA et al., 2016; ALVIM et al., 2016). Citrus blackfly eggs are creamy white until the second day after laying, resemble curved rods, with spiral-shaped, concentrating on the abaxial surface of leaves, adhered by a short peduncle. Usually on the seventh day after laying, eggs are orange, while on the eighth day, longitudinal hatching lines are

Received 02.08.17 Approved 08.21.17 Returned by the author 09.16.17
CR-2017-0092.R2

observed. Average incubation period lasts 15 days and mean viability is 88% at 27.4±1.1°C and 79.4±4.6% RH (PENA et al., 2009). Bioprospecting is essential for discovering alternative biotechnological solutions aiming to produce botanical insecticides (SACCARO JÚNIOR, 2011). The objectives of this study were to determine the content of secondary metabolites (carotenoids, flavonoids, phenolic compounds, and tannins) of *Argemone mexicana* L., *Ipomoea carnea* Jacq. subsp. *Fistulosa* (Martius ex Choisy), *Amorimia rigida* (A.Juss.) W. R. Anderson, *Ricinus communis* L. and *Syzygium aromaticum* (L.) Merr. & L. M. Perry, and evaluate the bioactivity of the ethanolic extracts on citrus blackfly eggs.

MATERIALS AND METHODS

The exsiccates of the studied plants were deposited and registered in the Universidade Estadual de Santa Cruz (UESC) Herbarium: 19126 (Mexican poppy), 19128 (pink morning glory), 19127 (amorimia), 18835 (castor bean) and 18821 (clove tree).

Phytochemical bioprospecting of the plant extracts was carried out at the Laboratory of Natural Products and Organic Synthesis (LPPNS), at the UESC, Ilhéus, Bahia. Total phenolic compounds, total flavonoids, condensed tannins, and carotenoids were quantified using UV-VIS (Nova® 1600UV) spectrometry of the leaves of Mexican poppy, pink morning glory, amorimia, and castor bean, and the peduncle of the clove flower buds. All analyses were performed in triplicates. Plants were dried in the shade and shredded in a blender. We used 0.2g of plant material for extraction with 50mL of acetone (KIMURA & RODRIGUES-AMAYA, 2003) for the determination of carotenoids. After clarification with $ZnSO_4$ and Ba $(OH)_2$, the compound was subjected to extraction with petroleum ether, and the carotenoid content was determined spectrophotometrically at 450nm. For flavonoids, we extracted 0.25g of plant material with methanol (PEIXOTO et al., 2008). Subsequently, we added glacial acetic acid, 2% pyridine methanolic solution, and $AlCl_3$. After 30 min, the spectrophotometric analysis was performed at 420nm. A calibration curve with 10, 30, 50, 70 and 100μg mL^{-1} methanolic solutions of rutin was prepared from the standard stock solution (5000μg mL^{-1}). This curve was represented by the following equation f (y) = 0.0075 x + 01, r^2 = 0.9349; the limit of detection was 0.480μg mL^{-1} and the limit of quantification was 1.60μg mL^{-1}. For determining the phenolic content, we extracted 0.3g of the plant material with ethanol (FURLONG et al., 2003). After clarification with $ZnSO_4$ and Ba $(OH)_2$, the extract was reacted with Folin-Ciocalteou and Na_2CO_3. After 30 minutes of reaction, the spectrophotometric reading was performed at 773nm. A calibration curve was prepared using 5, 10, 72.5, 145.0, and 217.5μg mL^{-1} ethanolic solution of gallic acid, which were was prepared from the standard stock solution (5000μg mL^{-1}). This curve was represented by the equation f (y) = 0.0034 x – 0.0194; r^2=0.9995; the limit of detection was 1.97μg mL^{-1} and the limit of quantification was 5.0μg mL^{-1}. For the determination of condensed tannins, we extracted 0.125g of plant material with 80% aqueous acetone solution, using a protocol adapted from PEREZ et al. (1999). The extract reacted with vanillin and concentrated HCl. After 20min, the spectrophotometry was performed at 500nm. A calibration curve with 5, 35, 65, 95 and 125μg mL^{-1} standard catechin solution was prepared from the stock solution of 140μg mL^{-1}. This curve was represented by the equation f (y) = 0.0024 x + 0.2173, r^2 = 0.9990; the limit of detection was 0.23μg mL^{-1} and the limit of quantification was 5.0μg mL^{-1}.

To evaluate the bioactivity of treatments, Pera sweet orange [*Citrus sinensis* L. Osbeck] leaves with eggs were kept in Petri dishes with moistened filter paper and sealed with plastic film. Each sample unit consisted of 30 eggs of citrus blackfly. Leaf areas containing the eggs were demarcated (VIEIRA et al., 2013). Eggs used in these experiments were laid no longer than 24h earlier, and had a creamy white appearance. The plant extracts were prepared from shredded dry material, 10g of each species were removed and placed in an Erlenmeyer flask containing 100mL of absolute alcohol for 8d. Thereafter, they were strained on filter paper and the pure ethanolic extracts (100%) were kept in amber glass. In order to prepare the treatments, pure ethanolic extracts were diluted with distilled water to 0.5, 1.0, 5.0, and 10% concentrations. Additionally, we used the commercial product Bioneem© diluted at the same concentrations. Distilled water was used as a control. Three applications of each treatment (n=7) were made by leaf immersion (30s per leaf). The application interval was 7s. Subsequently, the Petri dishes containing the leaves with eggs were closed, sealed with plastic film, identified, and allocated in BOD incubators (25±1°C; RU 60±5% and 12h of photophase). Egg mortality was evaluated weekly under a stereoscopic microscope (56×) after the first application of the treatments. Eggs that did not hatch until the 21st day were considered non-viable (JESUS et al., 2013, LIMA et al., 2013; VIEIRA et al., 2013).

RESULTS AND DISCUSSION

Phytochemical bioprospecting

Data on carotenoid, flavonoid, and phenolic compounds of *I. carnea*, *R. communis*, *A. mexicana*, *A. rigida*, and *S. aromaticum* are presented in figure 1. *S. aromaticum* showed high phenolic content, possibly due to the presence of eugenol, a volatile phenylpropanoid. It is the main compound repored in the steam of this species, as are β-caryophyllene (3.61%) and eugenyl acetate (3.76%), whereas α-humolene (0.60%) is a minor component (AFONSO et al., 2012). Further, the tannin content of this species was high (Figure 1). In the other species, negative values were obtained for tannin content, probably because the extracts were based on leaves (Figure 1). Phenolic compound level of *I. carnea* was 228.7mg g^{-1} (Figure 1). The phenolic constituents were not identified, but some studies have reported that the catechol content is between 45 and 75mg g^{-1} and flavonoid levels are high (SAHAYARAJ & RAVI, 2008; KHATIWORA et al., 2010; JAIN et al., 2016). Among the species evaluated in this study, *R. communis* had the highest flavonoid content (Figure 1). Moreover, presence of glycosides, quercetin, empferol, and kaempferol, which are phenolic compounds, have been reported in other studies (CHEN et al., 2008; PACHECO-SÁNCHEZ et al., 2012). The high content of tannins and phenolic compounds in *S. aromaticum*, and the high content of flavonoids in *R. communis* may be associated to the insecticidal potential of these species.

Bioassays

Except for the extract of *A. mexicana*, all the treatments had significant differences in citrus blackfly egg survival at 10% concentration (Table 1). Bioneem© caused more than 90% egg mortality (Table 1), and a

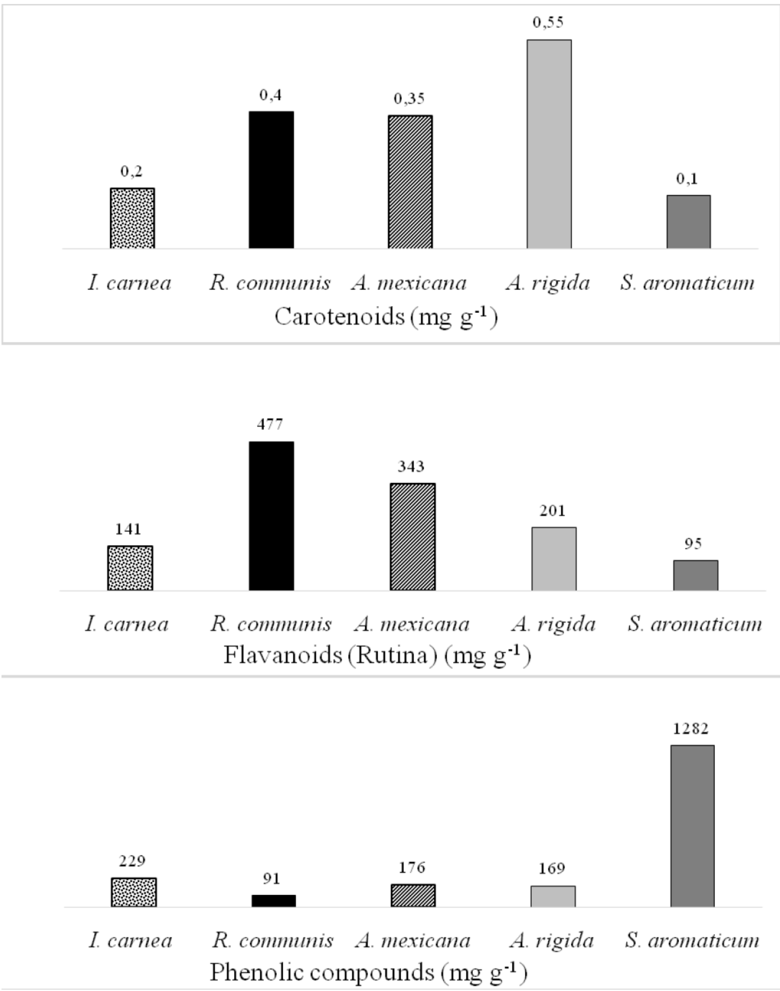

Figure 1 - Carotenoid, flavonoid (rutin), and phenolic compound content (mg g^{-1}) of the different plant species.

Table 1 – *Aleurocanthus woglumi* egg mortality (%) under different treatments, after 21 days of application in laboratory (25 ± 1°C; RH 60 ± 5% and 12h of photophase).

Treatments	0.5%	1.0%	5.0%	10.0%
I. carnea	65.5% ± 4.7 bB	64.5% ± 8.1 bcB	77.6% ± 6.9 Ba	79.1% ± 6.81 bA
R. communis	60.2% ± 5.14 bC	78.4% ± 13.79 bB	76.4% ± 14.24 bB	93.8% ± 16.6 bA
A. mexicana	68.1% ± 9.82 bA	74.1% ± 8.29 bA	71.9% ± 14.07 bcA	71.9% ± 6.14 bcA
A. rigida	66.9% ± 13.54 bA	65.5% ± 15.94 bA	74.99% ± 19.78 bA	77.2% ± 24.37 bA
S. aromaticum	78.2% ± 10.76 bAB	67% ± 3.24 bB	83.9% ± 10,2 bA	88% ± 29.33 bA
Bioneem©	94.1% ± 19.13 aA	94.3% ± 28.12 aA	96.6% ± 14.20 aA	97.2% ± 13.88 aA
Control	44.3% ± 3.6 cA	39.5% ± 3.39 cA	49.9% ± 6.8 cA	56% ± 3.56 cA

Values followed by the same uppercase letters on the rows and lowercase letters in a column do not differ statistically from each other, according to Tukey's honestly significant difference (HSD) test at the 5% probability level.

control efficiency of more than 80% at all concentrations (Figure 2), probably due to the action of azadirachtin and other compounds present in leaves and fruit of the neem tree (MARTINEZ, 2003). After leaf immersion in Bioneem©, 100% of citrus blackfly eggs darkened. Moreover, the commercial product Rot-Nim® (0.5%) has been reported to cause citrus blackfly egg mortality of 80.2% (SILVA et al., 2012), which indicated that neem-based products can efficiently control citrus blackfly. Extract of *S. aromaticum* (5 and 10% only) caused egg mortality higher than 80% (Table 1) and control efficiency of greater than 80% at 10% concentration (Figure 2), possibly due to the presence of eugenol (phenolic compound) and flavonoids (AFONSO et al., 2012). The highest control efficiency (81.6%) for the extract of *R. communis* was observed at 10% concentration (Figure 2), causing eggs mortality above 90% (Table 1). These results are supported by VIEIRA et al. (2013), who reported a decrease in the viability of citrus blackfly eggs as the commercial castor oil concentration increased. The extract of *A. rigida* caused citrus blackfly egg mortality higher than 70.0% at 5% and 10% concentrations only (Table 1). However, this species had low control efficiency (Figure 2). Furthermore, *A. mexicana* caused citrus blackfly egg mortality lower than 80.0% at all concentrations, which

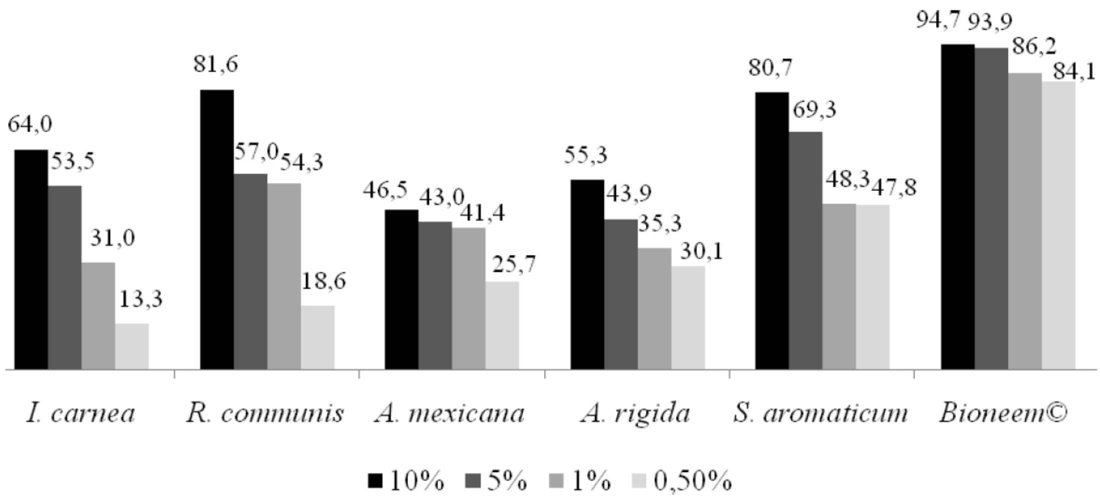

Figure 2 - Efficiency (%) of different treatments based on plant species on egg mortality of *Aleurocanthus woglumi* after three applications by leaf immersion (NAKANO; SILVEIRA NETO; ZUCCHI, 1981).

is similar to the results obtained for *A. rigida* species (Table 1, Figure 2). MARTÍNEZ-TOMÁS et al. (2015) reported that the aqueous extract of *A. mexicana* applied to eggs of the whitefly (Hemiptera: Aleyrodidae) caused a 97.64% reduction of the nymph population. Citrus blackfly egg viability is approximately 65-95% (PENA et al., 2009), which justifies 40% mortality in the control (Table 1).

CONCLUSION

The species *A. rigida*, *R. communis*, and *S. aromaticum* presented higher levels of carotenoids, flavonoids, and phenolic compounds, respectively. The commercial oil Bioneem© is effective in controlling citrus blackfly eggs. Ethanolic extracts of *S. aromaticum* and *R. communis* have insecticidal potential on citrus blackfly eggs, acting as alternative control agents for this species, besides subsidizing and contributing to its integrated management.

ACKNOWLEDGMENTS

The authors are grateful for the support of the Embrapa Cassava and Tropical Fruits and the Programa de Pós-graduação em Produção Vegetal (PPGPV).

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Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

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