Emirates Journal of Food and Agriculture. 2020. 32(8): 583-590
doi: 10.9755/ejfa.2020.v32.i8.2135
http://www.ejfa.me/

RESEARCH ARTICLE

Secondary metabolites of *Asclepias curassavica* (Apocynaceae) and its effects on food preference and mortality of *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

Renato Marcos de Leão[1], João Vitor Souza Cruz[1], Vânia Maria Ramos[1]*, Viviane Tavares de Almeida[1], Pedro Henrique Gorni[2], Roberto da Silva Camargo[1], Ana Cláudia Pacheco[2], Letícia Vieira de Lima[1], Luiz Carlos Forti[3]

[1]Laboratory of Agricultural Entomology, Agronomy Department, College o f Agricultural Sciences, University of Western São Paulo [Universidade do Oeste Paulista] (UNOESTE), Presidente Prudente, São Paulo, Brazil, [2]Agronomy Department, College of Agricultural Sciences, University of Western São Paulo [Universidade do Oeste Paulista] (UNOESTE), Presidente Prudente, São Paulo, Brazil, [3]Laboratory of Social Insects-Pests, Vegetal Protection Department, São Paulo State University [Universidade Estadual Paulista] - Botucatu (SP), Brazil

ABSTRACT

Asclepias curassavica L. plant is toxic for vertebrates, and little is known about its effects on invertebrates, as well as whether its secondary metabolites have an influence on food preference and survival of insects. Thus, a study was conducted to verify the action of *A. curassavica* on *Spodoptera frugiperda* J.E.Smith. The plants were collected, dried and ground to compose a crude ethanolic extract. Food tests were carried out with and without opportunity of choice, using corn leaf discs immersed in the extract at concentrations of 1%, 2% and 4%, for assessment of food consumption (g) and preference index. Later, the extract was topically applied on second- and fifth-instar caterpillars, at concentrations of 1%, 2%, 4% and 6%, in order to observe its effect on survival. In parallel, analysis was conducted to verify the presence and measure the amount of total polyphenols and flavonoids in the extract. There was contact action between the extract and second-instar caterpillars at all concentrations, with treatment 6% causing 100% of mortality 72 hours after application. None of the treatments promoted contact action on fifth-instar caterpillars. In food tests with and without opportunity of choice, all treatments caused reductions in consumption, which classified all as phagodeterrent, with treatment 4% standing out as the least favorite. The presence of total polyphenols and flavonoids was found in the extract, presenting 58.75 ug/mL and 150.1 ug/mL, respectively. The *A. curassavica* extract proved promising in *S. frugiperda* control.

Keywords: Bioinsecticides; Botanical insecticides; Corn pests; Fall armyworm; Scarlet milkweed

INTRODUCTION

Composed of around 490 species, genus *Asclepias* is widespread in the Paleotropical, Holarctic and Neotropical regions (Pereira et al., 2004), with some species having medicinal properties. For instance, *Asclepias curassavica* L. (Pinaceae) acts in the central nervous system and presents medicinal characteristics for treatment of rheumatism, tumors, inflammations and eye infections in mammals (Bernal and Correa, 1989; Li et al., 2009; Duke and Vasquez, 1994). However, its effects on insects are still contradictory. There are reports that *A. curassavica* diluted in ethanol is highly effective in controlling *Nomophila* sp caterpillars (Lepidoptera: Noctuidae) (Costa et al., 2014). On the other hand, no insecticidal activity has been observed for second and fourth-instar *Spodoptera frugiperda* J.E.Smith caterpillars, at 0.1% concentration (Macagnan et al., 2016).

In Brazil, it is estimated that genus *Spodoptera* (Lepidotera: Noctuidae) is responsible for most expenses with pesticides and losses that exceed 50% of damages caused by pests (Figueiredo et al., 2006). Traditional control of this insect is done with chemical products such as insecticides from organophosphate, carbamate and pyrethroid classes, which are highly toxic (Morillo and Notz, 2001).

***Corresponding author:**
Vânia Maria Ramos, Laboratory of Agricultural Entomology, Agronomy Department, College of Agricultural Sciences, Universidade do Oeste Paulista (UNOESTE), Rodovia Raposo Tavares, km 572, CEP 19067-175, Presidente Prudente, São Paulo, Brazil.
E-mail: vaniaramos@unoeste.br

Received: 28 February 2020; **Accepted:** 14 June 2020

Thus, population control methods for *S. frugiperda* are necessary in agricultural crops, resorting to a lower use of insecticides from the same chemical group or class and aiming at their alternation or substitution (Baskar et al., 2011). Substances with insecticidal activities that derive from the secondary metabolism of plants are promising and have been studied as new molecules for pest control (Menezes, 2014). Kim et al. (2003) reported that, for the rice weevil (*Sitophilus oryzae*), radish, mustard and cinnamon essential oils caused 100% mortality after the first day treatment, by direct contact, applying them on filter papers. Murugesan & Murugesh (2008) observed 87% reduction in the population of H*enosepilachina vigintioctopunctata* third day after spraying with neem oil. According to Menezes (2005) there are countless plants possessing insecticidal activity, and many of them need be studied and introduced, when possible, into agricultural properties as an alternative way of pest control.

Considering the harmful effects reported in the development of insects, *A. curassavica* offers a promising potential in agricultural pest control. Thus, the objective was to study the bioactivity of *A. curassavica* ethanolic extract on *S. frugiperda* under laboratory conditions, aimed at the development of a botanical insecticide.

MATERIAL AND METHODS

Spodoptera frugiperda farming

The insects came from a farming environment established in the Agricultural Entomology Laboratory of University of Western São Paulo (UNOESTE), Presidente Prudente city, São Paulo State, Brazil. In the farming, emerged moths were stored in plastic boxes (cages) measuring 32.5 x 37.5 x 55.5 (Height x Width x Length) and internally covered with filter paper, under temperature of 26.0°C± 1.0°C, relative humidity (RH) of 60±10% and photophase of 12 hours.

The moths were daily fed honey10%, which was placed in Petri dishes with cotton for capillary feeding. Lays were transferred to 75-mL capped plastic containers with artificial bean-based diet (Parra, 2001). The mass farming provided eggs and then, the caterpillars that were used in the experiment.

Extract Making

For extract sourcing, *A. curassavica* plants at reproductive stage were collected, stored in Kraft paper bags and dried in air-circulation oven at 60°C for 48h. They were then ground until 0.45-mm granulometry to obtain a fine powder that was stored in hermetically-sealed glass containers at 24°C, without lighting, until manipulation for the making of the extracts.

The powder obtained from the plant went through a maceration process for a week with an ethanol 96% solution for subsequent filtration. Filtration was done with the aid of a conventional glass funnel, using germination paper as filter. After filtration, the ethanol 96% was returned to the container until covering 4 cm of the volume filled by the powder. This procedure was repeated 7 times until exhaustion, when maximum color loss of the extract occurred during filtration, and the ethanolic extract was obtained (Santana et al., 2013).

After the complete filtration process, all the solvent was evaporated under reduced pressure in rotary evaporator to obtain the crude ethanolic extract of the scarlet milkweed.

The crude ethanolic extract was stored in hermetically-sealed glass containers wrapped with aluminum foil, at a temperature of 24°C, without lighting, where it remained until being used in the different assays. For the bioassays, 6,595 grams of *A. curassavica* green mass were collected, which later resulted in 1,522 grams of green mass, allowing a total extraction of 11.5 grams of crude ethanolic extract, that is, the extract yield in relation to fresh mass was 0.17%.

Food preference assays for S. frugiperda in relation to corn leaves immersed in A. curassavica ethanolic extract

Two tests were conducted in this bioassay: one with possibility of choice between substrate (corn leaf) treated with extract and non-treated substrate, and another without possibility of choice, using only substrate treated with the extract. These tests aimed to determine whether the extract had phagostimulant or phagodeterrent action on the insect.

For the assay, hybrid corn P3250 (PIONNER) was sown in 2-liter black bags containing soil (red latosol) and fertilizer, as per agricultural recommendations for the crop. The plants were grown in environmental chamber, model Fitotron SGC 120 Plant Growth Chamber, at a temperature of 21°C during the day, and 18°C during the night, where they stayed for 25 days; the leaves were then detached from the stem of the plant.

Discs of 7 mm in diameter were taken from the leaves with the aid of an iron hole cutter, and the discs were provided to the caterpillars, according to treatment. The solutions containing extract were prepared with ethanol 96% and crude extract at concentrations of 1%, 2% and 4%. The corn leaf discs were immersed in the respective solutions for 20 seconds, as per methodology by Kogan and Goeden (1970); afterwards, they were distributed on paper towel and maintained at room temperature for the solvent to evaporate and then provided to the caterpillars in the tests with and without possibility of choice.

The test with possibility of choice was carried out with Petri dishes measuring 25 cm in diameter, with the bottom covered with filter paper lightly moistened with distilled water. Corn leaf discs were arranged on the paper – 3 per dish: 1 disc (control) immersed in distilled water, 1 disc (control) immersed in ethanol 96%, and 1 disc (treatment) immersed in solution containing the extract at 1%, 2% or 4% (Fig. 1A). All discs were arranged in triangles and equidistant.

At the center of each dish, a second-instar caterpillar was released. After 24 and 48 hours, the caterpillars were taken away and the area of the discs was measured with the aid of software Imagem J. Leaf consumption, per insect, was obtained from the difference between the initial area of the leaf disc and the remaining area after the caterpillars ate. Each one of the treatments (1%, 2% and 4%) was represented by a set of 20 dishes, with each dish being one replicate.

The effect caused by the *A. curassavica* extract at different concentrations was assessed using the food preference index introduced by Kogan and Goeden (1970), based on equation PI = (2A/(M+A), where: PI = preference index; A = consumed area of treated discs; and M = consumed area of non-treated discs. PI values range between zero and two, being categorized as: phagostimulant effect, if PI is above 1; neutral effect, if PI is equal to 1; and phagodeterrent, if PI is below 1.

The assay without possibility of choice (Fig. 1B) was conducted and assessed just as the previous one, differing from the latter only for the provision of leaf discs from the same treatment, in each disc, to the caterpillars (distilled water; ethanol 96%, extract 1%, extract 2% or extract 4%).

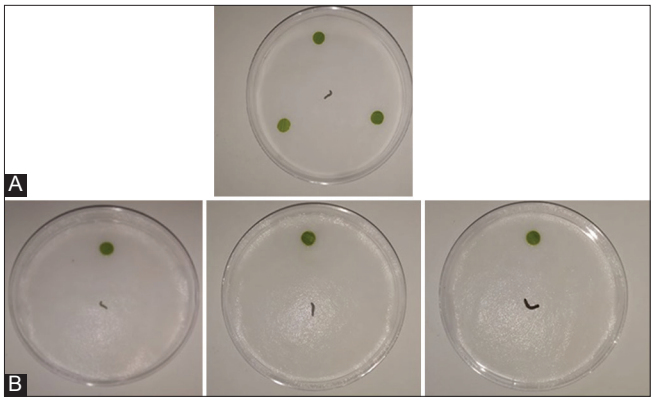

Fig 1. Food preference assays models for *S. frugiperda* in relation to corn leaves immersed in *A. curassavica* ethanolic extract. A - with possibility of choice (discs on the same dish, from left to right: water, ethanol 96%, extract at 1%, 2% or 3%). B – without possibility of choice (discs on separate dishes, from left to right: water, ethanol 96%, extract at 1%, 2% or 3%

Assays for contact action between A. curassavica ethanolic extract and S. frugiperda

To verify the contact action of *A. curassavica* ethanolic extract on the insects at different stages, two assays were conducted, one containing second-instar *S. frugiperda* caterpillars, and another with fifth-instar caterpillars. Different extract concentrations were assessed (0% or control; 1%, 2%, 4% and 6%); before use, the treatments containing extract were previously diluted in ethanol 96%. In both assays, caterpillars individualized in 75-mL plastic containers received, through a Hamilton syringe, a drop of approximately 0.1 μL of extract on their backs, once. After application of treatments, the caterpillars were inoculated in artificial diet in accordance with Parra (2001), and then observed at intervals of 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, 10 hours, 12 hours, 24 hours, 48 hours and 72 hours, when the number of dead individuals was counted. For each treatment, 20 caterpillars (replicates) of the respective assessed instar (second or fifth) were used.

Analysis of A. curassavica secondary metabolites

Flavonoids: The methodology to determine the amount of flavonoids present in the *A. curassavica* extract was applied in accordance with Yao et al. (2013). The samples contained 0.100 mL of extract + 0.400 mL of ethanol 70% + 0.050 mL of $NaNO_2$ (5%), with the "blank" containing everything but the extract. After 6 minutes, there was addition of 0.050 mL of $AlCl_3$ (10%) + 0.300 mL of NaOH (1 M) + 0.100 mL of water. Afterwards, the samples rested for 15 minutes in the dark. The absorbance used was of 510 nm. For the standard curve, a routine solution of 1,000 μg/mL was prepared and diluted for 25, 50, 75, 100, 250 e 500 μg/mL. Thus, the curve and equation of the line were determined, and the results were expressed as μg/mL. All analyses were performed in triplicates, and means were calculated.

Total polyphenols: To determine the amount of total polyphenols present in the *A. curassavica* extract, the methodology by Stagos et al. (2012) was adopted. For the standard curve, a gallic acid solution (1,000 μg/mL) was prepared and diluted for 25, 50, 75, 100, 250 and 500 μg/mL. The samples contained 25 μL of extract + 1,275 μL of distilled water + 125 μL of folic acid. After 3 minutes, there was addition of 350 μL of $NaCO_3$ + 750 μL of water. The "blank" contained everything but the extract. After rest of 1 hour, reading was done using the absorbance of 765 nm.

Statistical Analysis

For all assays, data were subjected to the Shapiro-Wilk normality test; then, with the normal data, the ANOVA test was used, comparing means by Tukey's test, and data that did not follow normal distribution were subjected to the Kruskall-Wallis non-parametric test for means

comparison, through program Action 2.9 (Estatcamp). In the food preference assays, analysis of variance was performed as well.

RESULTS

Secondary metabolites

The phytochemicals found in the *A. curassavica* crude ethanolic extract amounted to 58.75 ug/mL of total polyphenols and 150.1 ug/mL of flavonoids (Table 1).

Food preference

The interest of the caterpillars in the corn discs treated with different concentrations of *A. curassavica* ethanolic extract differed from control treatments in the test with possibility of choice (Table 1), indicating rejection by the caterpillars of discs containing the extract, in all treatments. Results show that the studied extract concentrations caused a phagodeterrent effect on *S. frugiperda*, reducing the interest of the caterpillars in the treated food. The treatment containing 4% of extract obtained the lowest absolute result, showing that in the 24 hours after the food was provided there was no consumption of treated corn discs, and 48 hours after, consumption was nearly null (Table 2). In addition to phagodeterrence, there was also a direct relationship between the increase in the concentration of *A. curassavica* extract and the decrease in the consumption of corn leaf area by *S. frugiperda* caterpillars (Table 2).

Table 1: Secondary metabolites present in *A. curassavica* ethanolic extract

Crude ethanolic extract	Total polyphenols	ug/mL	Flavonoids	ug/mL
Asclepias currassavica	+	58.75	+	150.1

+ present

In the assay without possibility of choice, all treatments with the extract also differed from control (Table 3) and, equally, there was an evident increase of rejection by the caterpillars of treated discs as the extract concentration increased. The treatment that presented lower leaf area consumption was 4%, with a reduction of 98% in relation to the control immersed in distilled water. There was also preference by the caterpillars for corn discs immersed in distilled water, compared to ethanol 96%.

Caterpillar mortality via topical extract application

In the assay with fifth-instar caterpillars, the treatments with extract did not differ from control, that is, none of the experimented extract concentrations caused the death of the caterpillars.

Differently, the second-instar caterpillars suffered the action of the extract applied topically (Table 4). After

Table 2: Consumption of non-transgenic corn (cm²), of leaves treated with different concentrations of A. curassavica ethanolic extract, by *S. frugiperda* caterpillars, with possibility of choice

Concentration	Treatment	Consumed area* (cm²) 24h	Consumed area* (cm²) 48h	Preference Index**	Classification***
0%	H20	0,324±0,026^{a}	0,568±0,040^{a}	1	Neutral
0%	ETHANOL	0,305±0,028^{a}	0,507±0,044^{a}	0,950	Phagodeterrent
1%	EXTRACT	0,011±0,011^{b}	0,034±0,023^{b}	0,113	Phagodeterrent
0%	H20	0,197±0,036^{a}	0,320±0,040^{a}	1	Neutral
0%	ETHANOL	0,178±0,035$_{a}$	0,303±0,037^{a}	0,970	Phagodeterrent
2%	EXTRACT	0,024 ±0,010^{b}	0,036±0,019^{b}	0,200	Phagodeterrent
0%	H20	0,293±0,03^{a}	0,423±0,043^{a}	1	Neutral
0%	ETHANOL	0,989±0,040^{b}	0,359±0,068^{a}	0,910	Phagodeterrent
4%	EXTRACT	0,000±0,000^{c}	0,011± 0,011^{b}	0,050	Phagodeterrent
*Y = 0,2091 $e^{-0,815x}$; R^2 = 0,8366					

Means followed by the same letter do not differ statistically from each other by the Kruskal-Wallis test at 1% level. **PI = 2A(M+A); where A = consumed area of treated discs, and M = consumed area of non-treated discs. ***Classification: phagostimulant, if the index is above 1; neutral, if equal to 1, and phagodeterrent, if below 1

Table 3: Consumption of non-transgenic corn (cm²), of leaves treated with different concentrations of *A. curassavica* ethanolic extract, by *S. frugiperda* caterpillars, without possibility of choice

Treatment	Concentration	Consumed area* (cm²) 24h	Consumed area* (cm²) 48h	Preference Index**	Classification***
H20	0%	0,381±0,003^{a}	0,741±0,019^{a}	1	Neutral
ETHANOL	0%	0,318±0,024^{b}	0,584±0,045^{b}	0,880	Phagodeterrent
T1%	1%	0,023±0,014^{c}	0,086±0,032^{c}	0,209	Phagodeterrent
T2%	2%	0,005±0,005^{c}	0,039±0,008^{c}	0.100	Phagodeterrent
T4%	4%	0,005±0,004^{c}	0,015±0,020^{c}	0,039	Phagodeterrent
*Y = 0,4457 $e^{-0,956x}$; R^2 = 0,8898					

Means followed by the same letter do not differ statistically from each other by the Kruskal-Wallis test at 1% level. **PI = 2A(M+A); where A = consumed area of treated discs, and M = consumed area of non-treated discs. ***Classification: phagostimulant, if the index is above 1; neutral, if equal to 1, and phagodeterrent, if below 1.

Table 4: Daily accumulated mortality (%) of second-instar *S. frugiperda* caterpillars after topical application of 0.1 µL/caterpillar of ethanolic extract of *A. curassavica* collected at reproductive stage

Treatment	Concentration	30 min	1h	2h	4h	6h	8h
H2O	0%	0±0^{c}	0±0	0±0^{b}	0±0^{b}	0±0^{b}	0±0^{b}
Ethanol	0%	10±0,67^{bc}	15±0,79^{c}	15±0,79^{b}	15±0,79^{b}	15±0,79^{b}	15±0,79^{b}
T1%	1%	45±0,111^{a}	45±0,111^{ab}	55±0,111^{a}	55±0,111^{a}	60±0,109^{a}	60±0,109^{a}
T2%	2%	45±0,111^{a}	65±0,106^{a}	65±0,106^{a}	65±0,106^{a}	70±0,102^{a}	70±0,102^{a}
T4%	4%	30±0,102^{ab}	35±0,106^{ab}	50±0,111^{a}	60±0,109^{a}	60±0,109^{a}	60±0,109^{a}
T6%	6%	25±0.096^{abc}	25±0.096^{bc}	55±0.111^{a}	75±0.096^{a}	80±0.089^{a}	85±0.079^{a}
Treatment	**Concentration**	**10h**	**12h**	**24**	**48h**	**72h**	
H2O	0%	0±0^{c}	0±0	0±0^{b}	0±0^{b}	0±0^{b}	
Ethanol	0%	10±0,79^{bc}	15±0,79^{c}	15±0,79^{b}	15±0,79^{b}	30±0,102^{b}	
T1%	1%	15±0,106^{a}	65±0,106^{ab}	70±0,112^{a}	85±0,079^{a}	85±0,079^{a}	
T2%	2%	65±0,102^{a}	75±0,096^{a}	75±0,096^{a}	75±0,096^{a}	75±0,096^{a}	
T4%	4%	70±0,106^{ab}	70±0,102^{bc}	70±0,102^{a}	70±0,102^{a}	85±0,079^{a}	
T6%	6%	85±0.079^{abc}	85±0.079^{bc}	85±0.079^{a}	85±0.067^{a}	100±0.000^{a}	

Means followed by the same letter do not differ statistically from each other by the Kruskal-Wallis at 1% level

30 minutes of application on the backs of the caterpillars, treatments 1% and 2% produced the best results in relation to the others, with 40% and 45% of mortality, respectively. Subsequently, starting 4 hours after application, treatment 6% proved superior to the others, registering 75% of mortality and keeping a higher percentage of dead caterpillars until the end of the assay. In the last assessment (72 hours after application), treatments with the extract did not differ from each other, but presented different values, with treatment 6% causing 100% of mortality, followed by 4% and 1%, with 85% of mortality, respectively, and, lastly, treatment 2%, with 75% of mortality. Controls water and ethanol, both different from extract treatments, did not differ from each other, but the former did not cause the death of the caterpillars, while the latter caused 30% of mortality.

DISCUSSION

Secondary metabolites

In the present study, the crude ethanolic extract of *A. curassavica* presented 58.75 ug/mL of total polyphenols and 150.1 ug/mL of flavonoids, agreeing with Jawale (2014), who, analyzing the same plant, also found the presence of alkaloids, saponins, flavonoids, steroids and tannins. The three main chemical classes mentioned as having insecticidal and fungicide action are alkaloids, phenols and terpenes (Boulogne et al., 2012), and it has been reported that plants with larvicide activity contain compounds that can act in combination or independently (Ndung'U et al., 2004). In the present study, the presence of polyphenols and flavonoids may explain the larvicide action of the *A. curassavica* extract on *S. frugiperda*.

Matos et al. (2011) link the toxicity of this plant, especially in the aerial parts, to the presence of a number of glycosides, including asclepiadin, and glycosides are a common form of flavonoids (Forkmann et al., 1999). The rejection by *S. frugiperda* of corn leaves treated with the *A. curassavica* extract, verified in the present study, may also be related to the presence of flavonoids. According to Dakora (1995), flavonoids present great biocontrol potential, with microbial and insecticidal activities, having repellency and food deterrence action.

Besides the ingestion action, flavonoids may also explain the contact action of the extract applied topically on the caterpillars because, according to Hostettmann and Marston (2005), contact insecticidal action on insects is due to the presence, in vegetal extracts, of several bioactive chemical products such as alkaloids, saponins, tannins, flavonoids and steroids.

We can also attribute the high larval mortality rate of *S. frugiperda* to the presence of polyphenols, reaching a value of 100% with the *A. curassavica* extract at 6%. Souza et al. (2013), assessing the aqueous extract of *Myracrodruo nurundeuva* at 5% and 10%, found mortality rates for *Zagreus bimaculosus* eggs ranging from 50% to 100%, respectively; for larvae and adults, these concentrations caused the mortality of only 20%. The authors attributed the observed mortality to the polyphenols and tannins of the plant, since, according to Harbone and Grayer (1994), *Myracrodruon urundeuva* plant is characterized by the presence of tannins and polyphenols, which protect the plants against herbivores and diseases.

Thus, botanical insecticides stand out as a viable alternative for pest control (Schoereder et al., 2012; Amoabens et al., 2014); this is due to some particularities, including their composition, which is a mix of active phytochemicals, which can reduce the evolution rate of resistance in comparison with the selective pressure exerted by unique pure toxins (Arnason et al., 1993), such as chemical

insecticides. Considering the chemical compounds present and the effects on *S. frugiperda*, *A. curassavica* extract stands out as a promising botanical insecticide.

Food preference

The food preference assay with possibility of choice showed rejection by the caterpillars to corn leaf discs treated with the *A. curassavica* ethanolic extract, in which all assessed concentrations caused a phagodeterrent effect on *S. frugiperda*, reducing the consumption activity of the caterpillars. This deterrence is connected to the sensorial mechanisms of the insect. The eating behavior of the insects depends on the integration of the central nervous system with chemoreceptors, located in the tarsi, in the mouthparts and in the oral cavity, and on substances found in plants with insecticidal activity, which may act on chemoreceptors, stimulating deterrent cells or blocking phagostimulants, inhibiting feeding (Mordue and Nisbet, 2000).

Other plants have also been studied and classified as to food preference among insects. For example, Poncio (2010), assessing the attractiveness of aqueous extracts at 10% w/v of *Azadirachta indica, Nicotiana tabacum*, *Melia azedarach, Eucalyptus citriodora, Cedrella fissilis, Trichilia claussenii, Blepharocalyx salicifolius, Eugenia uniflora, Cinnamomum camphora, Syzygium cuminii, Cymbopo gonnardus, Ateleia glazioviana, Ruta graveolens*, tested in *Brassica rapa* leaves provided to Microtheca ochroloma larvae, classified all treatments as phagodeterrent. Similarly, Lourenço (2016), assessing the effect of *Sotalia guianensis* essential oil at a concentration of 300 μL/15mL on third-instar *S. frugiperda* and *Anticarsia gemmatalis* larvae, verified consumption reductions of 59% and 66% in relation to non-treated corn leaf sections, respectively.

The relationship found between the concentration increase in the *A. curassavica* extract and the decreased consumption of corn leaf area by *S. frugiperda* caterpillars shows the importance of using not only an efficient product, but the proper amount too. In this study, the concentration of 4% proved more effective, as it prevented almost completely the caterpillars from eating. In agriculture, this result can help decrease damages caused to plants and, consequently, resulting losses. The damage is associated with the injury caused to the plants, and the injury is a consequence of feeding by the caterpillars, therefore, if the immediate objective is to reduce or stop the damage, the important thing is to avoid the injury (feeding) and not necessarily kill the insects instantly.

It is worth noting that tests with possibility of choice are important to prove whether treatments are indeed perceived by the insects, which then can decide not to eat treated leaves, given the option. In the field, this shows the importance of uniformly applying products with ingestion action during pulverization, in the sense of eliminating the possibility of choice to pests. If distribution failures occur in the application, the caterpillars will try to feed on the non-sprayed plant parts, reducing the efficiency of the operation, and, consequently, compromising the product.

In the assay without possibility of choice, results were similar: treatments containing extract were classified as phagodeterrent; there was decrease in leaf consumption with increased extract concentration, and the concentration of 4% was the most effective one in inhibiting consumption by the caterpillars. Mazzonetto et al. (2013), assessing the repellency of aqueous extracts at 10% w/v on *S. frugiperda* in corn leaves, in test without possibility of choice, observed that *Chenopodium ambrosioides, Corymbia citriodora* and *Chrysanthemum leucanthemum* extracts did not present significant difference in relation to control. This shows that different plants can produce different effects, even if on the same species of insect, making it necessary to study the effect of each plant on each species of insect, without possibility to generalize results.

This result opens up new possibilities in the management of *S. frugiperda* populations, because given the fact that caterpillars avoid plants treated with *A. curassavica* extract (4%) and choose to feed on untreated plants, we can think in a control method using trap plants, protecting the crop with the extract and attracting the pest to the other plants, where after concentrating they will be annihilated, in order to reduce the costs and the impact of a conventional application.

In both food preference assays, with and without possibility of choice, eating inhibition started soon in the first 24 hours after extract application, showing, from a practical point of view, that in the crop, even if the caterpillars do not die in the first hours after extract application, they immediately reduce or stop food consumption and, simultaneously, the damage caused to plants.

Caterpillar mortality via topical extract application

The results evidence that the extract used at the highest concentration (6%) was rather efficient in inducing the mortality of the insects (100%), creating new perspectives as to its utilization as insecticide in non-chemical pest control. These data are corroborated by Costa et al. (2014) while verifying the effectiveness of *A. curassavica* extract diluted in ethanol in a 1:10 proportion in the control of *Nomophila* sp caterpillar (Lepidoptera: Noctuidae), in which, after pulverization, the percentage of 0% of survival was determined, indicating the effectiveness of the extract.

S. frugiperda caterpillars have also proved susceptible to other plant species, such as *Trichilia claussenii*, which, topically applied on second-instar larvae, in the dose of 1 µL of solution, showed that the methanolic extract of the seeds caused 64% of mortality (Nebo et al., 2010). Contact insecticides are widely used in agriculture, and they become a very practical tool when the objective is to control pests that are exposed in the aerial part of plants, which represents a highly significant portion of agricultural pest species.

The same assay replicated with *S. frugiperda* caterpillars in the fifth-instar did not produce differences from treatments with extract in relation to controls, indicating that the susceptibility of the insects to the *A. curassavica* extract may be different, according to each life cycle phase. Similarly, Scott et al. (2005), while testing the insecticidal effect of *Piper nigrum* extract directly applied to infested turfs at a concentration of 20 mg.mL^{-1}, on *Rhizotrogus majalis*, known as turf pests, obtained positive mortality effect for second- and third-instar larvae; however, a further experiment required the application of 40 mg.mL^{-1} to cause significant mortality in third-instar insects.

Analyzing the set of results from the mortality assays, it is clear that the moment of application is imperative for the success of control, hence the importance of botanical insecticides becoming tools in programs for integrated pest management, in which periodical sampling of pests determines the right moment for control action. Considering the results of this experiment, if the objective is to kill the caterpillars via contact, the recommendation should be made for the second larval instar. When it comes to caterpillars, sampling is essential to determine the infestation and the average size of the caterpillars, ensuring the success of the operation carried out at the appropriate time.

CONCLUSION

The ethanolic extract of *A. curassavica* at reproductive stage presented total polyphenols and flavonoids, which promoted a phagodeterrent action on *S. frugiperda* caterpillars, in addition to causing their effective mortality in the second instar via topical application at 6% concentration.

ACKNOWLEDGEMENTS

LCF was the recipient of a fellowship granted by the National Council for Scientific and Technological Development [*Conselho Nacional de Desenvolvimento Cientifico e Tecnológico*] (Grant 301718/2013-0).

Author Contribution Statement

VMR and RML conceived and designed research, RML, JVSC, VTA and PHG conducted bioassays, JVSC and LVL colleted the plant material, VTA, ACP and LCF analyzed data and RSC and VMR wrote this manuscript. All authors read and approved the manuscript.

REFERENCES

Amoabeng, B. W., G. M. Gurr, C. W. Gitau and P. C. Stevenson. 2014. Cost: Benefit analysis of botanical insecticide use in cabbage: Implications for smallholder farmers in developing countries. Crop Prot. 57: 71-76.

Arnason, J. T., S. MacKinnon, M. B. Isman, T. Durst, B. J. R. Philogene, C. Hasburn, P. Sanchez, L. Poveda, L. S. Roman, C. Satasook, G. H. N. Towers, P. Wiriyachitra and J. L. MacLaughlin. 1993. Insecticides in tropical plants with non-neurotoxic modes of action. Recent Adv. Phytochem. 27: 107-131.

Baskar, K., S. Sasikumar, C. Muthu, S. Kingsley and S. Ignacimuthu. 2011. Bioefficacy of *Aristolochia tagala* Cham. against *Spodoptera litura* Fab. (*Lepidoptera*: *Noctuidae*). Saudi J Biol Sci. 18: 23-27.

Bernal, H. Y. and J. E. Correa. 1989. Espécies Vegetales Promisorias de los Paises del Convenio Andrés Bello. Secretaria Ejecutiva del convenio Andrés Bello, Bogotá, Guadalupe.

Boulogne, I., P. Petit, H. Ozier-Lafontaine, L. Desfontaines and G. Loranger-Merciris. 2012. Insecticidal and antifungal chemicals produced by plants: A review. Environ. Chem. Lett. 10: 325-347.

Costa, D. A. T., A. R. R. Sarno, F. A. F. Santos and L. Pasin. 2014. Efeito do extrato aquoso de *Asclepias Curassavic*a L. sobre o desenvolvimento e sobrevivência de lagartas. Rev. Cien. 6: 1-4.

Dakora, F. 1995. Plant flavonoids: Biological molecules for useful exploitation. Aust J Plant Physiol. 22: 87-99.

Duke, J. A. and R. Vasquez. 1994. Amazonian Ethnobotanical Dictionary. CRC Press, Boca Raton, Florida.

Figueiredo, M. L. C., A. M. P. Martins-Dias and I. Cruz. 2006. Relação entre a lagarta-do-cartucho e seus agentes de controle biológico natural na produção de milho. Pesq. Agropec> Bras. 41: 1693-1698.

Forkmann, G. and W. Heller. 1999. Biosynthesis of flavonoids. In: U. Sankawa, (Ed.), Comprehensive Natural Products Chemistry. Elsevier, Amsterdam, pp. 713-748.

Harborne, J. B. and R. J. Grayer. 1994. Flavonoids and insects. In: J. B. Harborne, (Ed.), The Flavonoids, Advances in Research since 1986. Chapman and Hall, London, pp. 589-618.

Hostettmann, K. and A. Marston. 2005. Chemistry and Pharmacology of Natural Products Saponins. Cambridge University Press, Cambridge, UK.

Jawale, C. S. 2014. Larvicidal activity of some saponin containing plants against the dengue vector *Aedes egypti*. Trends Biotechnol. Res. 1: 11-21.

Kim, S. I., J. Y. Roh, D. H. Kim, H. S. Lee and Y. J. Ahn. 2003. Insecticidal activities of aromatic plant extracts and essential oils against *Sitophilus oryzae* and *Callosobruchus chinensis*. J. Stored Prod. Res. 39: 293-303.

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

Li, J. Z., C. Qing, C. X. Chen, X. J. Hao and H. Y. Liu. 2009. Citoxicity of cardenolideos and cardenolide glycosidies from *Asclepias*

curassavica. Bioorg. Med. Chem. Lett. 19: 1956-1959.

Lourenço, A. M. 2016. Toxicidade e Repelência do Óleo Essencial de Negramina Para Lagarta do Cartucho e Lagarta da Soja. Ph.D. Thesis.

Macagnan, R., F. Werner and B. E. F. Rego. 2016. Eficácia de extratos vegetais no controle de *Spodoptera frugiperd*a (JE SMITH, 1797) em milho. Biosaúde. 14: 74-80.

Matos, F. J. A., H. Lorenzi, L. F. L. Santos, M. E. O. Matos, M. G. V. Silva and M. P. Sousa. 2011. Plantas Tóxicas: Estudo de Fitotoxicologia Química de Plantas Brasileiras. Instituto Plantarum de Estudo da Flora, São Paulo.

Mazzonetto, F., F. Coradini, R. Corbani and A. Dalri. 2013. Ação de inseticidas botânicos sobre a preferência alimentar e sobre posturas de *Spodoptera frugiperda* (JE Smith) (*Lepidoptera*: *Noctuidae*) em milho. EntomoBrasilis. 6: 34-38.

Menezes, C. W. G., W. D. S. Tavares, E. G. Souza and J. C. Zanuncio. 2014. Effects of crude extract fractions of *Adenocalymma nodosum* (*Bignoniaceae*) on duration of pupa stage emergence of *Tenebrio molitor* (*Coleoptera*: *Tenebrionidae*) and phytotoxicity on vegetable crops. Allelopathy J. 33: 141.

Menezes, E. L. A. 2005. Inseticidas Botânicos: Seus Princípios Ativos, Modo de Ação e Uso Agrícola. Embrapa Agrobiologia, Seropédica, Rio de Janeiro.

Mordue, A. J. and A. J. Nisbet. 2000. Azadirachtin from the neem tree *Azadirachta indica*: Its actions against insects. An. Soc. Entomol. Bras. 29: 615-632.

Morillo, F. and A. Notz. 2007. Resistencia de *Spodoptera frugiperda* (Smith) (*Lepidoptera*: *Noctuidae*) a lambdacihalotrina y metomil. Entomotropica. 16: 79-87.

Murugesan, N. and T. Murugesh. 2008. Efficacy of some plant products against Spotted Leaf Beetle (Hadda beetle), *Henosepilachna vigintiooctopunctata* (F.) in Brinjal. J. Biopest. 1: 67-69.

Nebo, L., A. Matos, P. Vieira, J. B. Fernandes, M. F. Silva and R. R. Rodrigues. 2010. Atividade inseticida dos frutos de *Trichilia claussenii* (*Meliaceae*) sobre *Spodoptera frugiperda*. Quim. Nova. 33: 1849-1852.

Parra, J. 2001. Técnicas de Criação de Insetos Para Programas de Controle Biológico. ESALQ/FEALQ, Piracicaba, SP.

Pereira, G. F., M. C.Valente, N. M. F. Silva and C. L. F. Ichaso. 2004. As plantas Apocinaceas-Asclepiadoideas. In: H. B. Rodrigues, (Ed.), Flora Ilustrada Catarinense. Herbario Barbosa Rodrigues, Itajai, SC, pp. 1-252.

Poncio, S. 2010. Bioatividade de Insecticidas Botánicos Sobre *Microtheca ochroloma* Stål (*Coleoptera*: *Chrysomelidae*). Ph.D. Thesis.

Santana, L. C. L., M. R. M. Brito, L. S. Lima, J. M. David, J. P. L. David and R. Freitas. 2013. Avaliação do potencial antioxidante, atividade antimicrobiana e antihelmíntica do extrato etanólico padronizado das folhas de *Mikania glomerata* Sprengel. Rev. Bras. Farm. 94: 120-129.

Schoereder, J. H., H. M. M. Silva, A. F. Carvalho and D. C. Muscardi. 2012. Proposed lime stone treatment as pest control fails for the leaf-cutting ant *Atta sexdens rubropilosa*. Crop. Prot. 42: 79-82.

Scott, I. M., N. Gagnon, L. Lesage, B. J. R. Philogène and J. T. Arnason. 2005. Efficacy of botanical insecticides from *Piper* species (*Piperaceae*) extracts for control of European chafer (*Coleoptera*: *Scarabaeidae*). J. Econ. Entomol. 98: 845-855.

Souza, J. I. R., C. R. F. Oliveira and C. H. C. Matos. 2013. Efeito do Extrato Aquoso de Aroeira Sobre Diferentes Fases do Predador *Zagreus bimaculosus*. Proceedings of the 13th Jornada de Ensino, Pesquisa e Extensão, Recife, Brasil.