BIOSCIENCE JOURNAL

INSECTICIDAL POTENTIAL OF ORGANIC EXTRACTS OF *Calotropis procera* TO *Spodoptera frugiperda*

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How to cite: SOUZA, T.A.N., et al. Insecticidal potential of organic extracts of *Calotropis procera* to *Spodoptera frugiperda*. *Bioscience Journal*. 2023, **39**, e39025. https://doi.org/10.14393/BJ-v39n0a2023-63699

Abstract

This study evaluated the toxic effects of organic extracts of *Calotropis procera* leaves on the survival, development, and reproduction of *Spodoptera frugiperda*. Solutions of crude methanol extract and hexane and methanol fractions of *C. procera* leaves were added at 1.15% and 2.14% concentrations to the artificial diet of *S. frugiperda*. The mortality and duration of larval and pupal phases, weights of female and male pupae, deformations of pupae and adults, the reduction of adults able to reproduce, pre-oviposition and oviposition periods, the number of postures per female, and the fecundity and fertility of *S. frugiperda* females were also evaluated. The extracts harmed the survival, development, and reproduction of *S. frugiperda*. The ingestion of extracts and fractions by caterpillars affected adults by decreasing the oviposition period, the number of postures, fecundity, and fertility. The crude MeOH extract at a 2.14% concentration harmed the evaluated parameters of the insect, except for pupal mortality, female pupae weight, and pre-oviposition period. The MeOH fraction at 2.14% caused a 50.0% mortality of caterpillars and 16.0% deformation in pupae and 33.0% in adults, reducing by 72.0% the population able to reproduce. The MeOH fraction at the 2.14% concentration caused 25.0% and 38.0% of pupal mortality and deformation, respectively. *Calotropis procera* has promising insecticidal properties for a biological insecticide, a convenient and sustainable strategy for protecting plants against *S. frugiperda*.

Keywords: Biological insecticide. Fall armyworm. Secondary metabolites. Silk cotton.

1. Introduction

The fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), is a highly polyphagous pest that infests several cultures. In Brazil, it is considered a key pest in maize (Jeger et al. 2017). It has a high diversity of hosts, which makes food availability throughout the year hard to control (Silva et al. 2017). In maize, *S. frugiperda* causes damage from the emergence to the formation of ears and may reduce production by 20% to 50% (Day et al. 2017; Feldmann et al. 2019).

Chemical control and the use of transgenic plants that express the Bt (*Bacillus thuringiensis*) gene have been the most common strategies for controlling the fall armyworm in Brazil. However, chemical insecticides have serious disadvantages compared to other alternative control methods because they can cause resurgence and resistance to pests, death of non-target organisms, residues in food, contamination of applicators, and environmental pollution (Tapondjou et al. 2002). Regarding the use of transgenic plants

in Brazil, the area planted with Bt maize can reach around 80.0% (Omoto et al. 2016). However, misusing this technology has harmed its effectiveness. For instance, not using refuge areas has helped develop the resistance of *S. frugiperda* populations to the insecticidal proteins of transgenics, threatening the sustainability of varieties of this culture (Farias et al. 2014; Horkoshi et al. 2016). Aiming to solve the problems of environmental contamination by chemical and resistance insecticides, researchers are currently seeking new control strategies for *S. frugiperda*.

Using plants with insecticide potential is among the strategies currently researched, based on prospecting secondary metabolites produced by plant species (Singhi et al. 2004; Bakavathiappan et al. 2012) so they can be used as alternatives to synthetic chemicals. Some of these metabolites are known as alkaloids, phenols, and terpenoids, which may present insecticidal activities on insects and cause repellency and/or food deterioration against various pests (Koul 2004).

Calotropis procera (Asclepiadaceae) is among the studied plants with secondary metabolite sources. This plant is popularly known in Brazil as silk cotton, silk flower, jealousy, jealousy cotton, milkweed, or burner. It is originally from India and Africa, with wide geographical distribution in tropical and subtropical regions. In the dry landscapes of the Brazilian hinterlands, silk cotton stands out for remaining green even in the most arid periods of the year. It was introduced in Brazil as an ornamental plant due to the beauty of its flowers. However, after entering the country, it was invaded by pastures due to high seed dissemination through the wind, which made it reach the northeast, midwest, and southeast regions. In semi-arid regions with poor soils and low rainfall levels, this plant shows good leaf mass production all year round (Melo et al. 2001; Andrade et al. 2005).

Studies indicate that silk cotton has insecticidal properties for several pests of economically significant crops, such as *Anticarsia gemmatalis* (Lepidoptera: Noctuidae), *Ceratitis capitata* (Diptera: Tephritidae), *Dysdercus peruvianus* (Hemiptera: Pyrrhocoridae) (Ramos et al. 2007), *Callosobruchus chinensis* (Coleoptera: Bruchidae) (Salunke et al. 2005), and *Lipaphis erysimi* (Hemiptera; Aphididae) (Arya et al. 2016).

There are studies on the toxic action of silk cotton in the form of aqueous extract on *S. frugiperda*, *C. capitata*, and *Henosepilachna elaterii* (Coleoptera: Coccinellidae) (Ahmed et al. 2006; Silva et al. 2015). Silk cotton is an invasive plant in Brazil, which makes it a compelling species for a natural insecticide in the form of an aqueous extract because of its abundant vegetation and labor as the only production cost for pest control use.

Studies have also shown that organic extracts of *C. procera* have a toxic action on *Spodoptera litura* (Lepidoptera: Noctuidae) (Bakavathiappan et al. 2012), *Trogoderma granarium* (Coleoptera: Dermestidae) (Khan et al. 2018), and *Helicoverpa armigera* (Lepidoptera: Noctuidae) (Lall et al. 2013). The toxic effect of organic extracts of *C. procera* on insect pests is probably due to the presence of secondary metabolites such as flavonoids (Heneidak et al. 2006; Srivastava et al. 2012), cardiac glycosides (Hanna et al. 2002), triterpenes (Bhutani et al. 1992), and sterols (Chundattu et al. 2016). Another possible explanation for the toxicity of this plant to insects is the abundance of latex produced in its green parts. Studies indicate that this latex can be produced to defend the plant against organisms such as insects, viruses, and fungi (Larhsini et al. 1997).

This study aimed to evaluate the toxic action of organic extracts of *C. procera* leaves on the survival, development, and reproduction of *S. frugiperda*.

The hypothesis is that organic extracts of *C. procera* leaves ingested in the larval stage of *S. frugiperda* harm pest biology.

2. Material and Methods

Biological tests were performed at the Entomology Laboratory of the State University of Montes Claros – UNIMONTES, Janaúba Campus – Minas Gerais, Brazil. *Spodoptera frugiperda* caterpillars were reared in the laboratory (25 ± 1°C, RH of 70 ± 10%, and photophase of 12 hours) and fed with an artificial diet (Greene et al. 1976). The organic components of *Calotropis procera* leaves were extracted at the Laboratory of Natural Products of the Department of Chemistry (DQI) of the Federal University of Lavras – UFLA.

Collection and preparation of plant material

Calotropis procera leaves were collected in the experimental area of UNIMONTES, Janaúba Campus, MG, Brazil (15° 49'56 '' south latitude, 43° 16'20 '' west longitude) on June 20, 2017, at the first hours of the day. New fully developed leaves were collected without apparent damage from diseases or insect pests. The fresh material (10.5 kg of leaves) was taken to the laboratory, where the collected leaves were distributed in brown paper bags (5.0 kg) with circular holes to allow air circulation and moisture to escape. The paper bags with the leaves remained in a forced air circulation oven regulated at 40°C for six days (144 hours) when they reached constant weight. After drying, the leaves were crushed in a Willey knife mill coupled to an 18-mesh sieve. The resulting powder was placed in a vacuum-sealed plastic bag and stored in a freezer for future use.

Preparation of the extract and fractions of *Calotropis procera* leaves

The organic extract and fractions were prepared with 1,000 g of powder from *C. procera* leaves. The powder was subdivided into four 250 g aliquots that were transferred to amber flasks with a capacity of 1,000 mL. Each flask received 500 mL of methyl alcohol (MeOH) over the powder. The flasks were manually shaken and remained at rest for 24 hours. After that, the mixtures were filtered through cotton wool. The resulting filtrates were transferred to amber-type flasks (1,000 mL), and each flask received another 400 mL of MeOH. After shaking, the flasks remained at rest for another 24 hours. The MeOH addition to the flasks, the 24-hour rest, and filtration were repeated six more times with a total of eight extractions.

All filtrates were combined and concentrated to dryness on a rotary evaporator with an initial pressure of ~ 135 mmHg and final pressure of ~ 15 mmHg, resulting in the crude methanol extract. This extract was divided into two subsamples of the same mass (38.26 g each): one was reserved for biological testing, and the other was used to continue the extractions.

One of the reserved subsamples was transferred to a 2,000 mL capacity beaker, which received 200 mL of hexane (Hex). This material was stirred on a magnetic stirrer for 10 minutes and filtered through cotton wool. The residue was subjected to three additional extractions with 200 mL of Hex each. The obtained filtrates were combined and concentrated to dryness on a rotary evaporator with an initial pressure of ~ 135 mmHg and final pressure of ~ 15 mmHg. This procedure resulted in the hexane fraction (Hex). The material that remained insoluble to Hex was washed four times with ethyl acetate (AcOEt), following the same procedure, to obtain a fraction soluble in AcOEt. The residue insoluble in AcOEt was subjected to four washes with MeOH by the same methodology. This procedure resulted in a fraction soluble in MeOH.

The extract and fractions were stored in glass flasks and remained refrigerated for later use. The AcOEt fraction was not used in biological tests with *S. frugiperda* due to the low production volume obtained in the extraction process.

Determination of the lethal concentration (LC) of the crude methanol extract (crude MeOH) to *Spodoptera frugiperda*

Initially, a standard solution of the crude MeOH extract (10%) was prepared. Hence, a glass beaker (50 mL) received 2.5 g of crude MeOH extract and distilled water plus Tween 80 at a 0.1% concentration until completing the volume of 25 mL. This solution was taken to a magnetic stirrer and then an ultrasound device. The solution remained for 30 minutes in each device. The other concentrations were obtained from the standard solution of the crude MeOH extract (10%) by adding distilled water plus 0.1% Tween 80. The concentrations were determined by calculating logarithmic interpolation between the lowest and highest values. Thus, the concentrations evaluated were 0.14%, 0.25%, 0.45%, 0.79%, 1.42%, 2.52%, and 4.50%.

For assessing the crude MeOH extract, artificial diet discs (1.0 cm in diameter x 0.6 cm in height) were individualized in Petri dishes (60 mm x 15 mm), and an aliquot (0.1 mL) of the crude methanol extract was pipetted over each at the defined concentrations. The control used diet discs treated with distilled

water or distilled water plus 0.1% Tween 80. On each diet disc, an *S. frugiperda* caterpillar was transferred and remained to feed for five days, when mortality was evaluated.

The research was performed in a completely randomized design with seven treatments (crude MeOH extract at concentrations of 0.14%, 0.25%, 0.45%, 0.79%, 1.42%, 2.52%, and 4.50%), two controls (distilled water and distilled water plus 0.1% Tween 80 solution), and 50 repetitions, each consisting of a five-day-old *S. frugiperda* caterpillar.

The homogeneity of variances for all variables was analyzed with the Bartlett test and the Residuals graph versus adjusted values for error normality. The results were submitted to regression analysis using the Sisvar Statistical Program, version 5.3 (Ferreira 2011).

Toxicity of the crude methanol extract of *Calotropis procera* leaves and fractions to *Spodoptera frugiperda*

Initially, standard solutions of crude MeOH extract and Hex and 10% MeOH fractions were prepared with the same previous procedure. The solutions were prepared at 1.15% and 2.14% concentrations for each standard by adding 0.1% Tween 80 aqueous solution, corresponding to the respective 40% and 70% lethal concentrations of the crude MeOH extract.

To evaluate the action of the crude MeOH extract and Hex and MeOH fractions on *S. frugiperda*, 0.1 mL of the solutions was pipetted onto the artificial diet discs (1.0 cm in diameter and 0.6 cm in height). The discs were individualized in flat-bottomed glass tubes (8.5 cm x 2.5 cm), and 24 hours after treatment, a five-day-old *S. frugiperda* caterpillar was transferred to them. The controls used diet discs treated with distilled water and distilled water plus 0.1% Tween 80. The glass tubes were plugged with cotton wool.

Spodoptera frugiperda caterpillars remained on the treated artificial diet for five days. Then, the surviving caterpillars were transferred to new glass tubes containing the untreated artificial diet and remained until pupation. The formed pupae were sexed, weighed, and individualized in glass tubes until the emergence of adults. The adults that emerged from each treatment (assessed concentrations and controls) were used to form couples. These couples were grouped in PVC cages (5 cm in diameter x 8 cm in height) covered with bond paper to allow laying by females. A Petri dish (90 mm x 15 mm) was placed at the cage base, and a piece of voile fabric was placed at the top of the tube. The adults were fed with a 10% honey solution and water.

The egg masses were collected daily and transferred to Petri dishes (60 mm x 15 mm) that were lined with filter paper moistened with deionized water, aiming at caterpillar outbreak. After hatching, the number of caterpillars and unviable eggs was recorded.

Mortality and duration of the larval phase, pupal mortality, duration and weight of female and male pupae, deformation percentage of pupae and adults, reduction percentage of adults able to reproduce, pre-oviposition and oviposition periods, the number of postures per female, fecundity, and fertility were evaluated.

The experiment was performed in a completely randomized design (DIC) with six treatments (three extracts and two concentrations), two controls (distilled water and 0.1% Tween 80 aqueous solution), and 20 replicates, each including an *S. frugiperda* caterpillar.

The homogeneity of variances for all variables was analyzed with the Bartlett test and the Residuals graph versus the adjusted values for error normality. The variables that did not meet the assumptions were subjected to Kruskal-Wallis analysis at a 5% probability using Action Stat software, version 3.5 (Team Estatcamp 2014). The variables that met the assumptions were subjected to the Scott-Knott test at a 5% probability using Genes software (Cruz 2013).

3. Results

The larval mortality results from the concentration adjustments of the crude MeOH extract of *C. procera* by the regression analysis indicated the linear model ($p < 0.001$; $\hat{Y} = 20.772564 + 14.533279x$) as the best fit for the data (Figure 1). The determination coefficient ($R2 = 86.51\%$) indicates a satisfactory

adjustment of the regression line, demonstrating the high strength measure of the relationship between the evaluated variables.

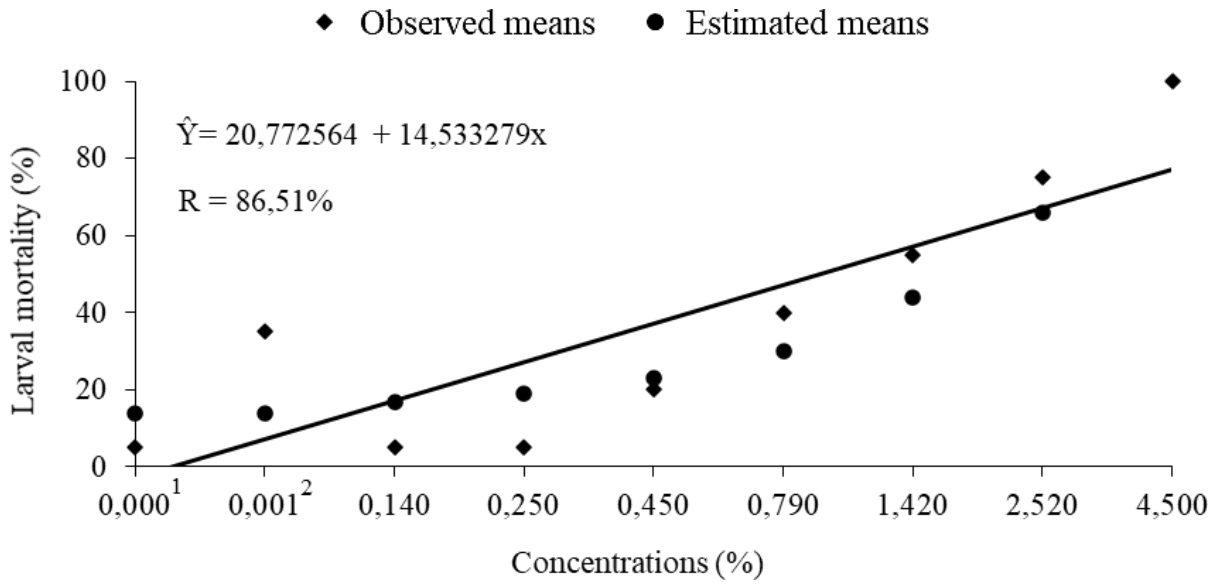

Figure 1. Larval mortality of *Spodoptera frugiperda* fed with an artificial diet treated at different concentrations of crude methanol extract of *Calotropis procera* leaves. [1] Control 1- Diet treated with distilled water; [2] Control 2 - Diet treated with 0.1% Tween 80 aqueous solution.

The increased mortality of *S. frugiperda* caterpillars depended on the concentration of the extract ingested by the insect along with the artificial diet. The highest mortality values occurred when the caterpillars ingested a diet with 2.52% and 4.50% concentrations of the crude MeOH extract, causing the death of 75.0% and 100.0% of insects, respectively. The lethal concentrations LC40, LC50, LC70, and LC90 estimated for the crude MeOH extract for caterpillars were 1.15%, 2.01%, 2.14%, and 4.76%, respectively.

The crude MeOH extract and fractions of *C. procera* leaves were toxic to *S. frugiperda*, with significant effects on larval (X^2 = 20.78; p <0.00411) and pupal (X^2 = 27.72; p <0.00015) mortality (Table 1). Crude MeOH extract ingestion (2.14%) killed 50.0% of caterpillars, higher than the controls (water and Tween), the crude MeOH extract (1.15%), and the MeOH fraction (1.15%). MeOH fraction ingestion (2.14%) by the caterpillars harmed the pupae, which died at a higher percentage than those of the controls, the crude MeOH extract (2.14%), and the Hex fraction (1.15%).

Table 1. Larval and pupal mortality (%), larvae duration (days), and male and female pupae of *Spodoptera frugiperda* after the caterpillars ingested an artificial diet treated at different concentrations of crude methanol (MeOH) extract of *Calotropis procera* leaves and their hexane (Hex) and methanol (MeOH) soluble fractions.

Treatment	Larval phase		Pupal phase		
	Mortality[1]	Duration[1]	Mortality[1]	Duration	
				Male [1]	Female[1]
Water	20.0 ± 5.71 b	14.8 ± 0.10 d	0.0 ± 0.00 b	8.9 ± 0.15 c	8.9 ± 0.17 b
Tween 80 aqueous solution	22.0 ± 5.91 b	15.7 ± 0.07 d	0.0 ± 0.00 b	8.2 ± 0.14 c	8.4 ± 0.10 b
Crude MeOH (1.15%)	22.0 ± 5.91 b	22.1 ± 0.20 a	13.2 ± 5.42 ab	13.0 ± 0.27 ab	11.8 ± 0.32 a
Crude MeOH (2.14%)	50.0 ± 7.14 a	22.5 ± 0.33 a	16.0 ± 0.00 b	13.0 ± 0.31 ab	11.6 ± 0.44 a
Soluble fraction in Hex (1.15%)	24.0 ± 6.10 ab	21.4 ± 0.14 bc	2.6 ± 2.63 b	13.0 ± 0.37 ab	11.3 ± 0.32 a
Soluble fraction in Hex (2.14%)	38.0 ± 6.93 ab	21.9 ± 0.24 ab	12.9 ± 6.12 ab	13.9 ± 0.27 a	12.4 ± 0.22 a
Soluble fraction in MeOH (1.15%)	18.0 ± 5.48 b	21.2 ± 0.12 c	14.6 ± 5.58 ab	12.6 ± 0.30 b	11.1 ± 0.24 a
Soluble fraction in MeOH (2.14)	30.0 ± 6.54 ab	21.9 ± 0.27 abc	25.7 ± 7.49 a	12.9 ± 0.40 ab	11.4 ± 0.45 a
χ^2	20.78	205.89	27.72	90.07	87.54

[1]Means followed by the same letter in the columns do not differ by the Kruskal-Wallis test at a 5% probability.

The ingestion of crude methanol extract and its fractions by *S. frugiperda* caterpillars also affected larval duration (X^2 = 205.89; p <0.00015) and pupal duration of female (X^2 = 87.54; p <0.00015) and male (X^2 = 90.07; p <0.00015) insects (Table 1). All the evaluated extracts extended the larval duration of the insect relative to those in the controls. The caterpillars that ingested the 1.15% and 2.14% crude MeOH extracts had the longest larval periods, six and seven days longer, respectively, then the controls. The crude MeOH extract and its fractions, both at the lowest and highest concentrations, extended the pupal duration of males and females compared to the controls. Duration increased three to four days for female and four to five days for male pupae relative to the controls.

The weight of male *S. frugiperda* pupae was affected by the ingestion of crude methanol extracts and their fractions by caterpillars (F = 2.80; p <0.01) (Table 2). The harmful effect on male pupae was the weight reduction found in the treatments of crude MeOH (2.14% and 1.15%), Hex (2.14%), and MeOH fraction (2.14). The weight of female pupae was not affected by crude MeOH extracts and their fractions (F = 0.93; p >0.05).

Table 2. Weight (mg) of male and female pupae, deformation (%) of pupae and adults, and reduction of adults able to reproduce (%) of *Spodoptera frugiperda* after the caterpillars ingest an artificial diet treated at different concentrations of crude methanol (MeOH) extract of *Calotropis procera* leaves and their hexane (Hex) and methanol (MeOH) soluble fractions.

Treatment	Pupal phase			Adult	
	Male weight[2]	Female weight[2]	Def. [1]	Def.[1]	Red. adults able to reprod[1]
Water	268.7 ± 5.10 b	247.1 ± 5.42 a	0.0 ± 0.00 b	0.0 ± 0.00 c	20.0 ± 5.71 d
Tween 80 aqueous solution	251.3 ± 6.19 a	247.2 ± 4.92 a	0.0 ± 0.00 b	7.7 ± 4.32 bc	28.0 ± 6.41 cd
Crude MeOH (1.15%)	248.1 ± 5.94 a	238.4 ± 4.94 a	0.0 ± 0.00 b	2.9 ± 2.94 c	34.0 ± 6.76 cd
Crude MeOH (2.14%)	255.6 ± 7.87 a	240.4 ± 5.52 a	16.7 ± 7.48 a	33.3 ± 10.54 ab	72.0 ± 6.41 a
Soluble fraction in Hex (1.15%)	274.1 ± 5.66 b	253.6 ± 6.12 a	0.0 ± 0.00 b	21.6 ± 6.70 abc	40.0 ± 6.99 bcd
Soluble fraction in Hex (2.14%)	255.8 ± 4.04 a	242.2 ± 6.43 a	0.0 ± 0.00 b	18.5 ± 7.61 abc	56.0 ± 7.09 abc
Soluble fraction in MeOH (1.15%)	264.9 ± 4.74 b	253.6± 7.95 a	2.4 ± 2.43 b	14.7 ± 6.16 abc	42.0 ± 7.05 bcd
Soluble fraction in MeOH (2.14)	246.5 ± 11.97 a	244.5 ± 5.70 a	0.0 ± 0.00 b	38.5 ± 9.73 a	68.0 ± 6.66 ab
CV (%)	10.0	9.9	-	-	-
χ^2	-	-	33.73	30.47	49.33

[1]Means followed by the same letter in the columns do not differ by the Kruskal-Wallis test at a 5% probability; [2]Means followed by the same letter in the columns do not differ by the Scott Knott test at a 5% probability; Def. = Deformation.

Calotropis procera extracts and their fractions, when ingested by *S. frugiperda* caterpillars, affected the formation of pupae (X^2 = 33.73; p <0.00002) and adults (X^2 = 30.47; p <0.0006) (Table 2). The caterpillars that ingested the crude MeOH extract (2.14%) were the only ones that became deformed pupae. In adults, there were deformations in insects whose caterpillars fed on crude MeOH (2.14) and MeOH (2.14%) extracts. The most common defect in adults was poor wing formation.

The ingestion of crude MeOH extracts and their fractions by *S. frugiperda* caterpillars caused insect deaths and deformations, significantly reducing the percentage of adults able to reproduce (X^2 = 49.33; p <0.000001) (Table 2). The reduction in the rate of viable adults occurred in the treatments of crude MeOH, MeOH, and Hex at a 2.14% concentration.

The pre-oviposition (X^2 = 25.11; p <0.0007) and oviposition (X^2 = 56.08; p <0.000001) periods, the total number of postures (X^2 = 57.93; p <0.000001), fecundity (X^2 = 51.54; p <0.000001), and fertility (X^2 = 37.35; p <0.05) of *S. frugiperda* were significantly affected by the ingestion of *C. procera* leaf extracts and fractions by caterpillars (Table 3). The pre-oviposition period of females showed a toxic effect of extracts and fractions in the crude MeOH and Hex treatments at a 1.15% concentration, in which females took an extra day to start their postures compared to the controls. The oviposition period showed a decrease in the total number of laying and fecundity when caterpillars ingested a diet containing methanol extracts

and their fractions at both evaluated concentrations. The crude MeOH (1.15 and 2.14%), Hex (1.15%), and MeOH (2.14%) extracts caused the highest reductions in *S. frugiperda* female fertility.

Table 3. Pre-oviposition and oviposition periods (days), the total number of postures, fecundity, and fertility (%) of *Spodoptera frugiperda* after the caterpillars ingested an artificial diet treated at different concentrations of crude methanol (MeOH) extract of *Calotropis procera* leaves and their hexane (Hex) and methanol (MeOH) soluble fractions.

Treatment	Pre-oviposition period[1]	Oviposition period[1]	Number of postures[1]	Fecundity[1]	Fertility[1]
Water	3.0 ± 0.17 bc	6.3 ± 0.22 c	9.7 ± 0.52 b	1.384.2 ± 52.94 b	98.7 ± 0.17 bc
Tween 80 aqueous solution	2.8 ± 0.16 c	6.3 ± 0.37 c	9.6 ± 0.65 b	1.374.3 ± 46.15 b	98.9 ± 0.18 c
Crude MeOH (1.15%)	4.1 ± 0.29 a	3.3 ± 0.25 ab	3.1 ± 0.22 a	628.9 ± 49.40 a	93.9 ± 1.05 a
Crude MeOH (2.14%)	4.0 ± 0.30 ab	3.1 ± 0.14 ab	3.3 ± 0.28 a	475.6 ± 42.84 a	94.7 ± 1.14 a
Soluble fraction in Hex (1.15%)	4.3 ± 0.31 a	3.3 ± 0.16 ab	3.3 ± 0.16 a	490.5 ± 38.01 a	95.6 ± 0.50 a
Soluble fraction in Hex (2.14%)	3.7 ± 0.35 abc	2.7 ± 0.18 a	2.7 ± 0.18 a	459.7 ± 49.38 a	96.2 ± 0.94 ab
Soluble fraction in MeOH (1.15%)	3.3 ± 0.16 abc	3.4 ± 0.17 b	3.3 ± 0.16 a	466.6 ± 25.66 a	96.8 ± 0.86 abc
Soluble fraction in MeOH (2.14%)	3.5 ± 0.16 abc	3.2 ± 0.13 ab	3.0 ± 0.00 a	474.7 ± 14.77 a	93.2 ± 1.26 a
χ^2	25.11	56.08	57.93	51.54	37.35

[1]Means followed by the same letter in the columns do not differ by the Kruskal-Wallis test at a 5% probability.

4. Discussion

This research showed that the increased mortality of *S. frugiperda* caterpillars depended on the concentration of the crude methanol (MeOH) extract of *C. procera* leaves added to the diet and ingested by the insect. Based on the toxic action of the crude MeOH extract of silk cotton on caterpillars, this study evaluated the LC40 and LC70 of the crude methanol extract and its fractions for pest survival, development, and reproduction. The mortality of *S. frugiperda* caterpillars after ingesting the crude MeOH extract of *C. procera* leaves may have been due to the secondary metabolites in this plant species.

Studies on the phytochemical composition of *C. procera* identified cardenolides, steroids, tannins, glycosides, phenols, terpenoids, sugars, flavonoids, alkaloids, and saponins as components of the leaves of this plant (Begum et al. 2010; Murti et al. 2010; Shrivastava et al. 2013). Phenolic compounds, terpenoids, and alkaloids identified in the leaves are the most common secondary metabolites in plant species with insecticidal activity (Boulogne et al. 2012).

Nicotine is among the most popular alkaloids, protecting plants in wild tobacco species against the attack of herbivorous insects such as *Spodoptera exigua* (Lepidoptera: Noctuidae), *Diabrotica undecimpunctata* (Coleoptera: Chrysomelidae), and *Trimerotropis* spp (Orthoptera) (Steppuhn et al. 2004; Steppuhn and Baldwin 2007). The phenolic compounds applied to *Capsicum annuum* (Solanaceae) leaves inhibited the feeding of *S. litura* caterpillars and affected their growth and development (Movva and Pathipati 2017). Saponins were also relevant secondary metabolites identified in *C. procera*. According to Chaieb (2010), the physiological mechanism responsible for saponin poisoning in insects is the potential interaction with cholesterol. Cholesterol is a precursor to ecdysteroid hormones, a class of insect growth regulators responsible for ecdysis. Hence, saponins can alter insect growth, cause failures in ecdysis, and

extend larval stages. This phenomenon may explain the elongation of the larval stage of *S. frugiperda* in the present study.

Secondary metabolites of plants represent a new generation of green insecticides with high potential for commercial use in agriculture (Dayan et al. 2009; Adeyemi 2010). A significant part of secondary metabolites found in plants is poorly soluble or insoluble in water, limiting their practical use as crop protection agents in the form of aqueous extracts. This limitation also appears in prospecting studies for substances with insecticidal action in plants. Therefore, research on organic extracts is vital.

This research verified the toxic action of aqueous extracts of silk cotton leaves on *S. frugiperda* (Silva et al. 2015). Hence, to continue these studies, extractions were performed with solvents in increasing order of polarity, such as hexane, ethyl acetate (unpublished data), and methanol, to obtain organic extracts. According to Jadhav et al. (2009), the solubility of different natural compounds in plants varies according to the solvents used for extraction. The polar solutes in plants are soluble in polar solvents such as methanol, ethanol, water, etc. The non-polar solutes in plants dissolve better in non-polar solvents such as hexane. The solubility of natural compounds usually increases with higher polarity indices.

This study found that the crude MeOH extract and fractions of silk cotton leaves caused the death of *S. frugiperda* caterpillars and pupae, lengthened the larval and pupal duration of males and females, reduced the weight of male pupae, and caused deformations in pupae and adults. They also harmed relevant stages of *S. frugiperda* adults, such as the pre-oviposition and oviposition periods, the number of postures per female, and the fecundity and fertility of females. It is worth noting that these harmful effects on the insect in this research were related to caterpillar ingestion of extracts and fractions at 40% and 70% concentrations, which are well below the LC100 of crude MeOH when insect mortality might be 100%. The toxic effects on the insect could be much more drastic at such higher concentration. Complementarily, the choice of LC40 and LC70 concentrations of the crude MeOH extract to *S. frugiperda* aimed to study the effects of ingesting underdoses of the extracts and their fractions by the caterpillars to obtain surviving insects and investigate the action of these substances in pest growth, development, and reproduction.

This study showed that the intake of a diet containing the crude MeOH extract at a 2.14% concentration by *S. frugiperda* caterpillars harmed the insect, verified in all the analyzed variables. These toxic effects were not observed only in pupal mortality, female pupal weight, and the pre-oviposition period. This may mean that the secondary metabolites in *C. procera* species with insecticidal action could all be carried away by methanol, which was efficient for this purpose. The results of this study corroborate Cechinel Filho and Yunes (1998), who indicated methanol solvent as the most suitable for obtaining crude plant extract because it allows extracting more compounds from plants. These authors also informed that, afterward, the crude methanol extract must be submitted to a liquid-liquid partition process using solvents with increasing polarities, such as hexane, dichloromethane, ethyl acetate, and butanol, to purify the substances through their polarities. However, according to the findings of this study, using hexane and methanol in the purification process did not cause higher toxicity in *S. frugiperda* than with crude methanol.

5. Conclusions

Further research is required to verify the toxicity of *C. procera* against *S. frugiperda* in the field before considering commercial applications. However, according to our findings, the organic extracts of *C. procera* showed insecticidal action and harmed the biology of *S. frugiperda*. Therefore, *C. procera* can be a potential candidate for developing a biological insecticide, aiming at a compelling and sustainable strategy for protecting plants against *S. frugiperda*.

Authors' Contributions: SOUZA, T.A.N.: conception and design, acquisition of data, analysis and interpretation of data, drafting the article; ALVARENGA, C.D.: conception and design, critical review of important intellectual content; SOARES, D.P.: acquisition of data, analysis and interpretation of data; GIUSTOLIN, T.A.: conception and design, analysis and interpretation of data, critical review of important intellectual content. All authors have read and approved the final version of the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

Ethics Approval: Not applicable.

Acknowledgments: The authors would like to thank the funding for this study provided by the Brazilian agency CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil), Financial Code 001. The authors would like to thank The State University of Montes Claros for providing the laboratory and all the materials used in this research. The authors would like to thank Denilson Ferreira de Oliveira, responsible for the Natural Products Laboratory of the Chemistry Department at the Federal University of Lavras, and Viviane Aparecida Costa for leading and guiding the production process of plant extracts.

References

ADEYEMI, M.M.H. The potential of secondary metabolites in plant material as deterents against insect pests: a review. *African Journal of Pure and Applied Chemistry.* 2010, **4** (11), 243–246. https://doi.org/10.5897/AJPAC.9000168

AHMED, U.A.M., et al. Evaluation of insecticidal potentialities of aqueous extracts from *Calotropis procera* Ait. against *Henosepilachna elaterii* Rossi. *Journal of Applied Sciences.* 2006, **6** (11), 2466-2470. https://doi.org/10.3923/jas.2006.2466.2470

ANDRADE, M.V.M., et al. Fenologia da *Calotropis procera* Ait R. Br., em função do sistema e da densidade de plantio. *Archivos de Zootecnia.* 2005, **54** (208), 631-634.

ARYA, H., SINGH, B.R. and SINGH, K. Insecticidal activity of *Calotropis procera* leaves against mustard aphid *Lipaphis erysimi* (kalt) and its natural predator *Coccinella septempunctata* (Linn). *Research Journal of Chemical and Environmental Sciences.* 2016, **4** (5), 53-55.

BAKAVATHIAPPAN, G., et al. Effect of *Calotropis procera* leaf extract on *Spodoptera litura* (Fab.). *Journal of Biopesticides.* 2012, **5**(Supplementary), 135-138.

BEGUM, N., SHARMA, B. and PANDEY, R.S. Evaluation of insecticidal efficacy of *Calotropis procera* and *Annona squamosa* ethanol extracts against *Musca domestica*. *Journal of Biofertilizers and Biopesticides.* 2010, **1** (1), 1-6. https://doi.org/10.4172/2155-6202.1000101

BHUTANI, K.K., GUPTA, D.K. and KAPIL, R.S. Occurrence of D/E trans stereochemistry isomeric to ursane (cis) series in a new pentacyclic triterpene from *Calotropis procera*. *Tetrahedron Letters.* 1992, **33** (49), 7593-7596. https://doi.org/10.1016/S0040-4039(00)60833-X

BOULOGNE, I., et al. Insecticidal and antifungal chemicals produced by plants: A review. *Environmental Chemistry Letters.* 2012, **10** (4), 325-347. https://doi.org/10.1007/s10311-012-0359-1

CECHINEL FILHO, V. and YUNES, R.A. Estratégias para a obtenção de compostos farmacologicamente ativos a partir de plantas medicinais: conceitos sobre modificação estrutural para otimização da atividade. *Química Nova.* 1998, **21** (1), 99-105. https://doi.org/10.1590/S0100-40421998000100015

CHAIEB, I. Saponins as insecticides: a review. *Tunisian Journal of Plant Protection.* 2010, **5** (1), 39-50.

CHUNDATTU, S. J., AGRAWAL, V. K. and GANESH, N. Phytochemical investigation of *Calotropis procera*. *Arabian Journal of Chemistry.* 2016, **9** (1), 230-234. https://doi.org/10.1016/j.arabjc.2011.03.011

CRUZ, C.D. Programa Genes: Biometria**.** Viçosa/MG. Editora UFV, 2013.

DAY, R., et al. Fall Armyworm: Impacts and Implications for Africa. *Outlooks on Pest Management.* 2017, **28** (5), 196-201. https://doi.org/10.1564/v28_oct_02

DAYAN, F.E., CANTRELL, C.L. and DUKE, S.O. Natural products in crop protection. *Bioorganic & Medicinal Chemistry.* 2009, **17** (12), 4022-4034. https://doi.org/10.1016/j.bmc.2009.01.046

FARIAS, J.R., et al. Geographical and temporal variability in susceptibility to Cry1F toxin from *Bacillus thuringiensis* in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) populations in Brazil. *Journal of Economic Entomology.* 2014, **107** (6), 2182-2189. https://doi.org/10.1603/EC14190

FELDMANN, F., RIECKMANN, U. and WINTER, S. The spread of the fall armyworm *Spodoptera frugiperda* in Africa—What should be done next? *Journal of Plant Diseases and Protection.* 2019, **126** (2), 97-101. https://doi.org/10.1007/s41348-019-00204-0

FERREIRA, D.F. Sisvar: a computer Statistical analysis system. *Ciência e Agrotecnologia.* 2011, **35** (6), 1039-1042. https://doi.org/10.1590/S1413-70542011000600001

GREENE, G.L., LEPPLA, N.C. and DICKERSON, W.A. Velvet bean caterpillar: a rearing procedure and artificial medium. *Journal of Economic Entomology.* 1976, **69** (4), 488-497. https://doi.org/10.1093/jee/69.4.487

HANNA, A.G., et al. Structure of a calotropagenin derived artifact from *Calotropis procera*. *Magnetic Resonance in Chemistry.* 2002, **40** (9), 599-602. https://doi.org/10.1002/mrc.1057

HENEIDAK, S., et al. Flavonoid glycosides from Egyptian species of the tribe Asclepiadeae (Apocynaceae, Subfamily Asclepiadoideae). *Biochemical System and Ecology.* 2006, **34** (7), 575-584. https://doi.org/10.1016/j.bse.2006.03.001

HORKOSHI, R.J., et al. Effective dominance of resistance of *Spodoptera frugiperda* to Bt maize and cotton varieties: implications for resistance management. *Scientific Reports*. 2016, **6** (1), 34864. https://doi.org/10.1038/srep34864

JADHAV, D., et al. Extraction of vanillin from vanilla pods: A comparison study of conventional soxhlet and ultrasound assisted extraction. *Journal of Food Engineering.* 2009, **93** (4), 421-426. https://doi.org/10.1016/j.jfoodeng.2009.02.007

JEGER, M., et al. Pest categorisation of *Spodoptera frugiperda*. *EFSA Journal.* 2017, **15** (7), e04927. https://doi.org/10.2903/j.efsa.2017.4927

KHAN, S. A., et al. Insecticidal efficacy of wild medicinal plants, *Dhatura alba* and *Calotropis procera*, against *Trogoderma granarium* (Everts) in wheat store Grains. *Pakistan Journal of Zoology.* 2018, **51** (1), 289-294. https://doi.org/10.17582/journal.pjz/2019.51.1.289.294

KOUL, O. Biological Activity of Volatile Di-n-Propyl Disulfide from Seeds of Neem, *Azadirachta indica* (Meliaceae), to Two Species of Stored Grain Pests, Sitophilus oryzae (L.) and Tribolium castaneum (Herbst). *Journal of Economic Entomology.* 2004, **97** (3), 1142–1147. https://doi.org/10.1093/jee/97.3.1142

LALL, D., et al. Larvicidal effects of leaf powder of *Calotropis procera* and *Argimone mexicana* against 4th instar of american boll warm, *Helicoverpa Armigera* (Hubner) (Noctuidae: Lepidoptera). *International Proceedings of Chemical, Biological and Environmental Engineering.* 2013, **60** (24), 122-125. https://doi.org/10.7763/IPCBEE

LARHSINI, M., et al. Evaluation of antifungal and molluscicidal properties of extracts of *Calotropis procera*. *Fitoterapia*. 1997, **68** (4), 371-373.

MELLO, M.M., et al. Estudo fitoquímico da *Calotropis procera* Ait., sua utilização na alimentação de caprinos: efeitos clínicos e bioquímicos séricos. *Revista Brasileira de Saúde e Produção Animal*. 2001, **2** (1), 15-20.

MOVVA, V. and PATHIPATI, U.R. Feeding-induced phenol production in *Capsicum annuum* L. influences *Spodoptera litura* F. Larval growth and physiology. *Archives of Insect Biochemistry and Physiology.* 2017, **95** (1), e21387. https://doi.org/10.1002/arch.21387

MURTI, Y., YOGI, B. and PATHAK, D. Pharmacognostic standardization of leaves of *Calotropis procera* (Ait.) R. Br. (Asclepiadaceae). *International Journal of Ayurveda Research.* 2010, **1** (1), p. 14-17. https://doi.org/10.4103/0974-7788.59938

OMOTO, C., et al. Field-evolved resistance to Cry1Ab maize by *Spodoptera frugiperda* in Brazil. *Pest Management Science*. 2016, **72** (9), 1727-1736. https://doi.org/10.1002/ps.4201

RAMOS, M.V., et al. Performance of distinct crop pests reared on diets enriched with latex proteins from *Calotropis procera*: Role of laticifer proteins in plant defense. *Plant Science.* 2007, **173** (3), 349–357. https://doi.org/10.1016/j.plantsci.2007.06.008

SALUNKE, B.K., et al. Efficacy of flavonoids in controlling *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae), a post-harvest pest of grain legumes. *Crop Protection.* 2005, **24** (10), 888-893.

SHRIVASTAVA, A., SINGH, S. and SINGH, S. Phytochemical investigation of different plant parts of *Calotropis procera*. *International Journal of Scientific and Research Publications.* 2013, **3** (8), 1-4.

SILVA, H. D., et al. Bioatividade dos extratos aquosos de plantas às larvas da mosca-das-frutas, *Ceratitis capitata* (Wied.). *Arquivos do Instituto Biológico.* 2015, **82**, 1-4. https://doi.org/10.1590/1808-1657000132013

SILVA, D.M., et al. Biology and nutrition of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) fed on different food sources. *Scientia Agricola.* 2017, **74** (1), 18-31. https://doi.org/10.1590/1678-992x-2015-0160

SINGHI, M.V., JOSHI, R.C. and SHARMA, K.S. Oviposition behavior of *Aedes aegypti* in different concentrations of latex of *Calotropis procera*: Studies on refractory behavior and its sustenance across gonotrophic cycles. *Dengue Bulletin.* 2004, **28**, 184-188.

SRIVASTAVA, N., CHAUHAN, A.S. and SHARMA, B. Isolation and characterization of some phytochemicals from Indian traditional plants. *Biotechnology Research International.* 2012, e549850. https://doi.org/10.1155/2012/549850

STEPPUHN, A., et al. Nicotine's defensive function in nature. *PLOS Biology.* 2004, **2** (8), e217. https://doi.org/10.1371/journal.pbio.0020217

STEPPUHN, A. and BALDWIN, I.T. Resistance management in a native plant: Nicotine prevents herbivores from compensating for plant protease inhibitors**.** *Ecology Letters.* 2007, **10** (6), 499-511. https://doi.org/10.1111/j.1461-0248.2007.01045.x

TAPONDJOU, L. A., et al. Efficacy of powder and essential oil from *Chenopodium ambrosioides* leaves as post-harvest grain protectants against six-stored product beetles. *Journal of Stored Products Research.* 2002, **38** (4), 395-402. https://doi.org/10.1016/S0022-474X(01)00044-3

TEAM ESTATCAMP (2014). Software Action. Estatcamp - Consultoria em estatística e qualidade, São Carlos - SP, Brasil. URL: http://www.portalaction.combr/

Received: 22 October 2021 | **Accepted:** 12 April 2022 | **Published:** 6 April 2023