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Effect of extract of Amazon Moss on Spodoptera Frugiperda (JE Smith) (Lepidoptera: noctuidae) in maize

Bryophytes are a potential group for the discovery of biologically active substances to pest control. The objective of this study was to evaluate the antifeedant and insecticidal effect of the ethanolic extract of Sematophyllum subsimplex (Hedw). Mitt. on second-instar larvae of Spodoptera frugiperda (JE Smith, 1797) on corn (Zea mays L.) leaves. The bioassay involved placing individual larvae in petri dishes and fed circular corn leaf sections with 50 µl of the extract at concentrations of 2.0%, 1.0%, 0.5% and 0.25% µg/µl, and DMSO (control), with five replicates, in no-choice feeding assays. Herbivory and mortality rates were assessed 24, 48 and 72 hours after application of the extract. The concentration of 0.25 µg/µl had a positive antifeedant effect shortly after 24 hours. All tested concentrations caused high mortality rates, suggesting insecticidal activity. This is the first scientific work to report the antifeedant and/or insecticidal activity of S. subsimplex and can be the basis for botanical insecticide formulation.

Keywords: Antifeedant activity; Botanical insecticide; Bryophytes; Insect control.

Efeito do extrato de Musgo Amazônico sobre Spodoptera Frugiperda (JE smith) (Lepidoptera: noctuidae) em milho

As briófitas são um grupo potencial para a descoberta de substâncias biologicamente ativas para o controle de pragas. O objetivo deste estudo foi avaliar o efeito antifeedant e inseticida do extrato etanólico de Sematophyllum subsimplex (Hedw). Mitt. em larvas de segundo instar de Spodoptera frugiperda (JE Smith, 1797) em folhas de milho (Zea mays L.). O bioensaio envolveu a colocação de larvas individuais em placas de petri e alimentadas com seções circulares de folhas de milho com 50 µl do extrato nas concentrações de 2,0%, 1,0%, 0,5% e 0,25% µg/µl, e DMSO (controle), com cinco repetições, em ensaios de alimentação sem escolha. As taxas de herbivoria e mortalidade foram avaliadas 24, 48 e 72 horas após a aplicação do extrato. A concentração de 0,25 µg/µl teve um efeito antifeedant positivo logo após 24 horas. Todas as concentrações testadas causaram altas taxas de mortalidade, sugerindo atividade inseticida. Este é o primeiro trabalho científico a relatar a atividade antifeedant e/ou inseticida de S. subsimplex e poderá servir como base para a formulação de inseticida botânico.

Palavras-chave: Atividade antifeedant; Inseticida botânico; Briófitas; Controle de insetos.

Topic: **Desenvolvimento, Sustentabilidade e Meio Ambiente**

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INTRODUCTION

Corn (*Zea mays* L.) is of great socioeconomic importance worldwide for its low cost, high nutritional value in human and animal diet, viability for small- and large-scale cultivation, and because it is the basis of several agro-industrial chains. Corn crops are, however, attacked by various pests, involving several species which cause different levels of damage and economic losses and are controlled by different techniques (GALVÃO et al., 2014). Among them, the fall armyworm *Spodoptera frugiperda* (SMITH, 1797) (Lepidoptera: Noctuidae) plays an important role. This insect has a recurrent and wide geographical distribution and potential to cause significant worldwide economic damages (SÁ et al., 2009; BARROS, 2012). The average percentage loss depends on the development stage of the plant and the climate and may reach 15% at up to 30 days of development of the crop and between 37% and 60% in the flowering phase (CRUZ et al., 1982; CRUZ, 1995, 1997; CRUZ et al., 1999; SARMENTO et al., 2002; BARROS, 2012).

Brazil stands out as the world's third largest producer of corn, behind only China and the United States, with an average productivity of 5.2 t/ha (SOUZA et al*.,* 2017). Corn is the second most produced grain in the country, especially in the Midwest and South regions, where cultivation has occurred in a massive way, in recent years, during the regular harvest season, besides the off-season second cultivation called the "small harvest" (MIRANDA et al*.,* 2019). In general, the control of the fall armyworm in Brazil relies on non-judicious use of synthetic insecticides, disregarding the principles of Integrated Pest Management (IPM) (RODRÍGUEZ et al., 2001) and consequently contributing to the contamination of the environment, producers, and consumers, and to non-selective death of organisms and emergence of resistant insect species. In this scenario, substances derived from secondary plant metabolism come as alternative pest control agents that do not necessarily imply life-threatening danger, contamination of the environment (MEDEIROS, 1990; LANCHER, 2000) or the other problems mentioned above.

In the biotechnology industry, bryophytes represent a potentially valuable group for the discovery of biologically active substances that can be efficient in pest control. The progress of research on the potential of chemical constituents present in this plants comes from reports involving more than 1,000 species hitherto studied outside Brazil, with promising results for different biologicals activities (ASAKAWA, 1982, 1995, 1999, 2007, 2008, 2011, 2013; SABOVLJEVIĆ et al., 2001, 2006, 2010; FATOBA et al., 2003; FRAHM, 2004; XIE et al., 2009; ALAN et al., 2012; GLIME, 2017). The results have demonstrated a high diversity of substances that provide typical properties and aromas such as pungency and bitterness, as well as an extraordinary set of biological activities, such as antibacterial, antifungal, antioxidant, nematocidal, allelopathic, and insect antifeedant, with biotechnological and commercial potential.

Antifeedant and insecticidal effects of extracts of mosses and liverworts on larvae of the genus *Spodoptera* Guenée have been we sought to study the possible phagodeterent and/or insecticidal effect of metabolites of the moss *Sematophyllum subsimplex* Hedw. Mitt. (Sematophyllaceae), since other species of the family have shown antioxidant and antidiabetic activities (MUKHOPADHYAY et al*.,* 2013; TATIPAMULA et al*.,* 2017). It is known that the same or most chemical compounds can naturally occur, in different concentrations, in species of the same botanical family. The volatile chemical composition of *S. subsimplex*

has been studied, revealing significant quantities of sesquiterpenes, fatty acids and aldehydes (MORAES et al. S/D), which are chemical groups with confirmed biological activities. However, no record exists in the literature concerning the use of *S. subsimplex* extracts for the control of agricultural pests.

The above considerations point to the relevance of the present study, which aimed to direct efforts to bryophyte phytochemistry, a field not yet explored in Brazil as in the international context. The goal was to contribute with information on the efficiency of extracts of the moss *S. subsimplex* in the management of the fall armyworm. The antifeedant and insecticidal effect of different concentrations of the ethanolic extract of *S. subsimplex* on second-instar *S. frugiperda* larvae fed on corn leaves was evaluated under controlled conditions.

METHODOLOGY

Material was collected in the Gunma Ecological Park, an Amazonian Forest fragment located in the city of Santa Bárbara, Pará, Brazil (01°13'00.86'' S and 48°17'41.18'' W), with 400 ha of native tropical rainforest and 140 ha of open area for multiple use (ALMEIDA et al*.,* 2003). *Sematophyllum subsimplex* is adapted to different environments (generalist), occurring throughout the Amazon region (GRADSTEIN et al., 2001; SANTOS et al., 2003; FAGUNDES et al., 2016). Samples were collected in a non-flooded secondary forest, near a stream, and at the entrance of igapós (flooded forests), on the bark of living trees, using the techniques of Yano (1989). Voucher samples were deposited at the João Murça Pires Herbarium of the Emílio Goeldi Museum of Pará, in Belém, Pará, Brazil.

The identification and sorting of species was done by the first author of the study, a specialist in bryophytes, according to Waard (1996), and the taxonomic classification followed Goffinet et al. (2008). The screening process was carried out at the Laboratory of Briology in the Emílio Goeldi Museum of Pará. The botanical material was washed, sun-dried and kept in a refrigerated environment. Different bryophyte genera and species ofte grow intertwined in tufts, mats or clumps (RICHARDS, 1984); thus, specimens of the moss of interest were carefully separated with the aid of tweezers (Flume 5) under a magnifying glass (Leica, Wild M3Z) and whole plants (gametophytes) were used for extract preparation.

The extraction procedure was carried out at the Central Laboratory of Chemical Extraction of the Federal University of Pará (UFPA). A dry sample of *S. subsimplex* (4g was sufficient for the species) was weighed on a precision balance (SHIMADZU, model AY220), crushed in a blender and stored in an Erlenmeyer flask. The process consisted of three extractions by cold maceration with 95% ethanol (200 ml). Every two days the material was vacuum filtered and remacerated, renewing the extractive liquid only in order to ensure that the largest number of substances was extracted from the plant matrix, until completing a period of six days.

The crude extract (final volume) of each step was collected in a volumetric flask, in a rotary evaporator (Büchi brand, model R-3) under reduced pressure (45 °C ± 1 °C) to minimize possible degradation of the chemical constituents due to the action of high temperatures (SIMÕES et al., 2000; BARBOSA, 2004). The mass contained in the flask was taken to a laminar flow hood and the extractive

content was determined for the dry mass of 71.6 mg (yield obtained) of the ethanolic extract of *S. subsimplex*.

The ethanolic extract of the plant was diluted in dimethylsulfoxide (DMSO) and a stock solution was prepared using an extract mass/solvent volume ratio of 20mg/10ml. Dilutions (treatments) were prepared from this solution to obtain the concentrations of 2.0, 1.0, 0.5 and 0.25 µg/µl, according to Markham et al. (2006), with adaptations. In the control group, DMSO was diluted in distilled water.

Caterpillars were collected initially from a corn plantation near the Soil Department of the Federal Rural University of Amazonia (UFRA), and from plants cultivated in pots with standard fertilization and kept in greenhouse conditions. They were individually placed in transparent plastic boxes (gerbox) with dimensions of 12 x 12 x 3 cm and reared on natural diet (corn leaves) to obtain the initial colony of larvae.

The rearing procedure was carried out in the Laboratory of Insecticide Bioecology (LABIN/ICA/UFRA) in an air-conditioned environment at 24 ± 2 °C and 70 ± 10% relative humidity. After the larvae turned into pupae, they were transferred to new boxes with moistened filter papers on the bottom to maintain moisture. The adults (moths) were put in PVC (polyvinyl chloride) tube cages of 30 cm in diameter and 40 cm in height, internally coated with filter paper for laying.

Moths were fed a 10% honey solution on pieces of cotton. The laid eggs were removed by cutting the portion of paper with the egg mass and packed in sterilized gerbox boxes. The newly hatched larvae (first-instar) were transferred with a brush into PET-bottles (MAIA et al., 2004) adapted for caterpillars with fresh corn leaves. After the first instar, the larvae were transferred into disposable cups with the artificial diet proposed by Bowling (1967). The biological cycle lasts an average of 48 days (SALVADORI et al., 1982).

The bioassay was performed with 25 individualized second-instar caterpillars. This instar was selected in order to perform the control tests at the beginning of the larval phase, when caterpillars have a dietary preference for young leaves of the plant (HARRISON, 1984; HOY et al., 1987). Furthermore, it is important to consider that caterpillars' sensitivity to secondary compounds decreases as larval development progresses (BELLANDA et al., 2009). Focusing on this instar may make it possible to prevent the growth to later instars, phases in which the insects burrow into the growing points of the plant, infesting and damaging the spikes and causing yield losses (PARRA et al., 1995; GASSEN, 1996).

Maize leaf sections (2.3 cm diameter) were cut from 30- to 40-day-old plants with a scrapbook-type punch, preferentially from leaves from the middle of the plant, and washed in distilled water. Five treatments were applied, namely, 2.0, 1.0, 0.5 and 0.25 µg/ml, and control (DMSO diluted in distilled water). The treatments were prepared at the moment of use by diluting the extract (stock solution) in DMSO according to the abovementioned concentrations, based on literature (WADA et al., 1971; LABBÉ et al., 2005; MARKHAM et al., 2006). A 50-µl aliquot was added to each leaf disc with an automatic pipette according to the respective treatments.

After evaporation of the solvent, the leaves were transferred into 10 cm diameter Petri dishes, where second-instar larvae were inoculated. The Petri dishes were lined with moist filter paper discs and kept moist throughout the experiment to preserve the turgescence of the leaf discs.

The experimental design was completely randomized with five treatments and five replicates, with each replicate containing a second-instar larva (BORGONI et al., 2005).

The method adopted in this study to evaluate *S. frugiperda* larval viability under different concentrations of the ethanolic extract of *S. subsimplex* was the one described by Borgoni et al. (2005), Labbé et al. (2005), Castro et al. (2006), Markham et al. (2006), and Haines et al. (2009), with adaptations.

The biological parameters evaluated were herbivory and mortality of caterpillars at 24, 48 and 72 hours after the experiment was set up. A relative leaf damage scale (0 to 5), adapted from Markham et al. (2006), was used for quantitative comparisons and determination of the percentage of consumption of corn leaf discs. The scores 0, 1, 2, 3, 4 and 5 represent, respectively, 0%, 1-25%, 26-50%, 51-75%, 76-99%, and 100% of leaf damage. The mortality rate was estimated by counting the number of dead caterpillars per day, which were removed from the Petri dishes after confirmation of death.

The experimental data were recorded at 24, 48 and 72 hours and submitted to analysis of variance to determine the antifeedant activity, using the Mann-Whitney U test to compare means (SIEGEL, 1956). This test evaluates the significance of the differences between the control and each treatment and indicates possible differences between treatments. All tests were unilateral and a confidence level of $\alpha = 0.05$ was adopted.

The Fisher's test (SIEGEL, 1956) was applied to mortality data, with a confidence level $\alpha = 0.05$, to check the significance of the differences between frequencies of paired independent samples (control x each of the treatments). This test assumes that the number of dead caterpillars in the treatments is higher than in the control group. The Tocher's modification was used to verify the significance of slight differences (as in the case of the present design with small samples). Fisher's test with Tocher's correction represents the most powerful statistical option for data in 2 x 2 tables (SIEGEL, 1956). All analyses were performed in the BioEstat 5.0 software (AYRES et al*.,* 2007).

RESULTS AND DISCUSSION

Antifeedant activity

24-hour assessment

Differences were found in leaf consumption by caterpillars after 24 hours, as estimated by the sum of scores of T1, T2, T3, and T4 (Figure 1) in contrast with the control group, where 26% to 50% of herbivory was observed only in T1 (Table 1). There were significant differences between the control and the treatments (Mann-Whitney U test p-value = 0.0379 for T1, p-value = 0.0184 for T2, T3, and T4) (Table 1). The data indicated a feeding deterrent action of different concentrations of *S. subsimplex* extract on second-instar fall armyworm caterpillars after 24 hours of consumption. As for the differences in the percentage of herbivory, no differences between treatments were found (Table 1). Therefore, the application of the concentration of 0.25 µg/µl on second-instar caterpillars had satisfactory antifeedant results 24 hours after the experiment was set up.

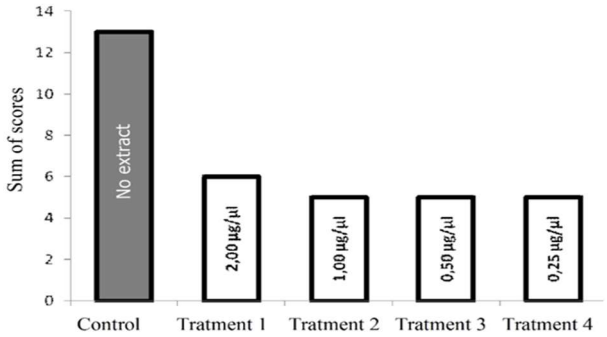

Figure 1: Summed scores of herbivory by second-instar *Spodoptera frugiperda* caterpillars submitted to treatments with different extract concentrations of *Sematoplhyllum subsimplex* (Hedw.) Mitt. 24 hours after the experiment was set up.

Table 1: Mann-Whitney U test results for different extract concentrations of the species *Sematophyllum subsimplex* applied to the food of second-instar *Spodoptera frugiperda* caterpillars, comparing the control group to each of the treatments and pairwise comparisons between treatments 24 hours after the experiment was set up.

	Control group comparisons with each of the treatments				**Comparisons between treatments**					
	Control group (no extract) vs. Treatment 1 (2.00 µg/µl)	Control group (no extract) vs. Treatment 2 (1.00 µg/µl)	Control group (no extract) vs. Treatment 3 (0.50 µg/µl)	Control group (no extract) vs. Treatment 4 (0.25 µg/µl)	Treat. 1 (2.00 µg/µl) vs. Treat. 2 (1.00 µg/µl)	Treat. 1 (2.00 µg/µl) vs. Treat. 3 (0.50 µg/µl)	Treat. 1 (2.00 µg/µl) vs. Treat. 4 (0.25 µg/µl)	Treat. 2 (1.00 µg/µl) vs. Treat. 3 (0.50 µg/µl)	Treat. 2 (1.00 µg/µl) vs. Treat. 4 (0.25 µg/µl)	Treat. 3 (0.50 µg/µl) vs. Treat. 4 (0.25 µg/µl)
Z statistics	1.7756	2.0889	2.0889	2.0889	0.5222	0.5222	0.5222	0.0000	0.0000	0.0000
Critical value	1.6449	1.6449	1.6449	1.6449	1.6449	1.6449	1.6449	1.6449	1.6449	1.6449
p-value	**0.0379**	**0.0184**	**0.0184**	**0.0184**	**0.3008**	**0.3008**	**0.3008**	**0.5000**	**0.5000**	**0.5000**

48-hour assessment

Little change occurred in leaf damage scores from 24 to 48 hours after the experiment was set up (Figure 2). No significant changes were observed in the feeding behavior of caterpillars, with overlaping results in T2, T3 and T4 (p-value = 0.0184). The group of caterpillars in T1 had slightly higher scores than those in the other treatments (p-value = 0.0236) (Table 2). It was also noticed that T1, T2, T3, and T4 presented significantly smaller scores than the control group and pairwise comparisons between treatments with the U test also showed no significant differences (Table 2). Hypothesis tests indicated that the superiority of the scores of T1 in relation to the other treatments were not significant (p-value = 0.3008) (Table 2).

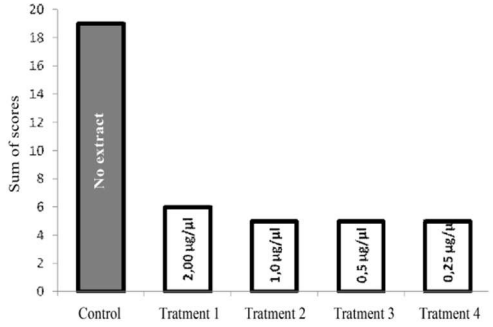

Figure 2: Summed scores of herbivory by second-instar *Spodoptera frugiperda* caterpillars submitted to treatments with different extract concentrations of *Sematoplhyllum subsimplex* (Hedw.) Mitt. 48 hours after the experiment was set up.

Table 2: Mann-Whitney U test results for different extract concentrations of the species *Sematophyllum subsimplex* applied to the food of second-instar *Spodoptera frugiperda* caterpillars, comparing the control group to each of the treatments and pairwise comparisons between treatments 48 hours after the experiment was set up.

	Control group comparisons with each of the treatments				**Comparisons between treatments**					
	Control group vs. Treat. 1 (2.00 µg/µl)	Control group vs. Treat.2 (1.00 µg/µl)	Control group vs. Treat. 3 (0.50 µg/µl)	Control group vs. Treat. 4 (0.25 µg/µl)	Treat. 1 (2.00 µg/µl) vs. Treat. 2 (1.00 µg/µl)	Treat. 1 (2.00 µg/µl) vs. Treat. 3 (0.50 µg/µl)	Treat. 1 (2.00 µg/µl) vs. Treat. 4 (0.25 µg/µl)	Treat. 2 (1.00 µg/µl) vs. Treat. 3 (0.50 µg/µl)	Treat. 2 (1.00 µg/µl) vs. Treat. 4 (0.25 µg/µl)	Treat. 3 (0.50 µg/µl) vs. Treat. 4 (0.25 µg/µl)
Z statistics	1.9845	2.0889	2.0889	2.0889	0.5222	0.5222	0.5222	0.0000	0.0000	0.0000
Critical value	1.6449	1.6449	1.6449	1.6449	1.6449	1.6449	1.6449	1.6449	1.6449	1.6449
p-value	**0.0236**	**0.0184**	**0.0184**	**0.0184**	**0.3008**	**0.3008**	**0.3008**	**0.5000**	**0.5000**	**0.5000**

72-hour assessment

The results of the Mann-Whitney U Test indicated that all treatments had significantly lowerscores than those in the control group (Figure 3). None of the treatments differed significantlyfrom the control, neither from each other, according to the pairwise comparisons between treatments (Table 3).

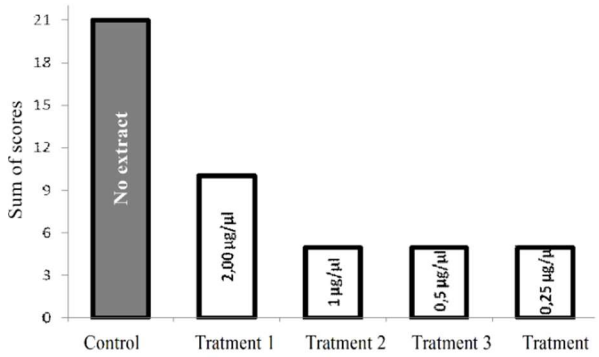

Figure 3: Summed scores of herbivory by second-instar *Spodoptera frugiperda* caterpillars submitted to treatments with different extract concentrations of *Sematoplhyllum subsimplex* (Hedw.) Mitt. 72 hours after the experiment was set up.

Table 3: Mann-Whitney U test results for different extract concentrations of the species *Sematophyllum subsimplex* applied to the food of second-instar *Spodoptera frugiperda* caterpillars, comparing the control group to each of the treatments and pairwise comparisons between treatments 72 hours after the experiment was set up.

	Control group comparisons with each of the treatments				**Comparisons between treatments**					
	Control group (no extract) vs. Treat. 1 (2.00 µg/µl)	Control group (no extract) vs. Treat. 2 (1.00 µg/µl)	Control group (no extract) vs. Treat. 3 (0.50 µg/µl)	Control group (no extract) vs. Treat. 4 (0.25 µg/µl)	Treat. 1 (2.00 µg/µl) vs. Treat. 2 (1.00 µg/µl)	Treat. 1 (2.00 µg/µl) vs. Treat. 3 (0.50 µg/µl)	Treat. 1 (2.00 µg/µl) vs. Treat. 4 (0.25 µg/µl)	Treat. 2 (1.00 µg/µl) vs. Treat. 3 (0.50 µg/µl)	Treat. 2 (1.00 µg/µl) vs. Treat. 4 (0.25 µg/µl)	Treat. 3 (0.50 µg/µl) vs. Treat. 4 (0.25 µg/µl)
Z statistics	1.8800	2.0889	2.0889	2.0889	1.0445	1.0445	1.0445	0.0000	0.0000	0.0000
Critical value	1.6449	1.6449	1.6449	1.6449	1.6449	1.6449	1.6449	1.6449	1.6449	1.6449
p-value	**0.0301**	**0.0184**	**0.0184**	**0.0184**	**0.1481**	**0.1481**	**0.1481**	**0.5000**	**0.5000**	**0.5000**

Cumulative food consumption scores over 24, 48 and 72 hours showed that the herbivory rate increased significantly only in the control group. *Sematophyllum subsimplex* extract proved to be similarly

efficient in deterring feeding by caterpillars at all tested concentrations (Figure 4). The results of T2, T3 and T4 overlapped, and the group of caterpillars in T1 presented slightly higher scores than those in the other treatments, which indicates that larger extract concentrations promote a stronger antifeedant action. However, hypothesis testing indicated that the superiority of the scores of T1 in relation to the other treatments was not significant.

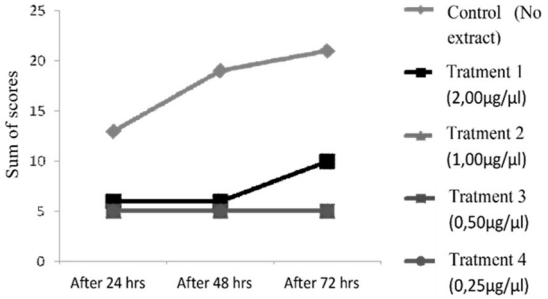

Figure 4: Evolution of cumulative herbivory scores by second-instar *Spodoptera frugiperda* caterpillars under different extract concentrations of *Sematophyllum subsimplex* over 24, 48 and 72 hours of consumption.

Mortality

24-hour assessment

The Fisher's test was not performed in the first observation because there was a dead caterpillar in T1 and in the control group. This test has a confidence level of $\alpha = 0.05$ and is unilateral, for it is assumed that the number of dead caterpillars in the treatments is higher than that in the control group. The Fisher's test was performed to verify the differences between T2, T3, and T4 in relation to T1, because in the latter there was a dead caterpillar while there was none in the others (Table 4). The results showed no significant differences in mortality between treatments (p-value [Fisher] = 0.5000). There were no significant variations in mortality after 24 hours of contact of the caterpillars with the food treated with *S. subsimplex* extract, thus indicating no insecticidal effect.

Table 4: Number of live and dead caterpillars observed in the T1 and the T2 or T3 or T4 after 24 hours of contact of second-instar fall armyworm with *Sematophyllum subsimplex* (Hedw.) Mitt. extract.

	Treat. 1 (2.00 µg/µl)	Treat. 2 or 3 or 4 (2.00 or 0.25 µg/µl)	Total
Live	4	5	**9**
Dead	1	0	**1**
Total	**5**	**5**	**10**
p-value (Fisher)	**0.5000**		
p-value (Tocher)	**0.5000**		

48-hour assessment

The results obtained in the control group were compared, using the Fisher's test, to those obtained in T1 and T2; and T3 or T4, for the latter two had the same number of dead caterpillars. The number of deaths in the control group and in T1 was statistically similar (p-value [Fisher] = 0.5000) (Table 5). The number of deaths in T2 was significantly higher than that in the control group (p-value [Fisher] = 0.0238), and insecticidal action was observed at the concentration of 1.00 µg/µl (Table 5). The number of deaths in T3 and T4 was significantly higher than that in the control group (p-value [Tocher] = 0.0238) (Table 5).

Insecticidal action was also observed at the concentrations of 0.50 or 0.25 µg/µl.

Table 5: Number of live and dead caterpillars observed in the control group and in the T1, T2 and T3 or T4 after 48 hours of contact of second-instar fall armyworm with Sematophyllum subsimplex (Hedw.) Mitt. extract.

	Control group comparisons with Treatment 1 and with Treatment 2						**Control group comparisons with Treatment 3 or 4**		
	Control (No extract)	Treat. 1 (2.00 µg/µl)	Total	Control No extract	Treat. 2 (1.00 µg/µl)	Total	Control (No extract)	Treat. 3 or 4 (0.50 or 0.25 µg/µl)	Total
Live	4	3	**7**	4	0	**4**	4	2	**6**
Dead	1	2	**3**	1	5	**6**	1	3	**4**
Total	**5**	**5**	**10**	**5**	**5**	**10**	**5**	**5**	**10**
p-value (Fisher)	**0.5000**			**0.0238**			**0.2619**		
p-value (Tocher)	**0.0833**						**0.0238**		

72-hour assessment

All treatments differed from the control group and T2 and T4 had the same number of dead caterpillars (100%). The number of deaths in T2 was significantly higher than that in the control group (p-value [Fisher] = 0.0238) (Table 6), and insecticidal action was observed at the concentration of 2.00 µg/µl. The number of deaths was significantly lower in the control group than in T2 or T4 (p-value [Fisher] = 0.0238) and T3 (p-value [Fisher] = 0.0040) (Table 6). Insecticidal action was observed at the concentrations 2.00, 0.5, 1.00 or 0.25 µg/µl.

Table 6: Number of live and dead caterpillars observed in the control group and in the T1, T2 and T3 or T4 after 72 hours of contact of second-instar fall armyworm with *Sematophyllum subsimplex* (Hedw.) Mitt. extract.

	Control group comparisons with Treatment 1 and Treatment 3						**Control group comparisons with Treatment 2 or 4**		
	Control (No extract)	Treat. 1 (2.00 µg/µl)	Total	Control (No extract)	Treat. 3 (0.50 µg/µl)	Total	Control (No extract)	Treat. 2 or 4 (1.00 ou 0.25 µg/µl)	Total
Live	4	2	**6**	4	1	**5**	4	0	**4**
Dead	1	3	**4**	1	4	**5**	1	5	**6**
Total	**5**	**5**	**10**	**5**	**5**	**10**	**5**	**5**	**10**
p-value (Fisher)	**0.2619**			**0.1032**			**0.0238**		
p-value (Tocher)	**0.0238**			**0.0040**					

The comparisons showed that mortality was statistically the same across treatments; T1, T2 (or T4) and T3 did not lead to different mortality rates of caterpillars (Table 7). All treatments had insecticidal effect. It can be said that the number of deaths in T2 or T4 was statistically equal to that in T1, as well as in T3.

The number of live caterpillars at 24, 48 and 72 hours differed significantly (p-value ≤ 0.05) in all evaluations, because the application of *S. subsimplex* extract to corn leaf discs led to lower survival rates of the insect compared to the control group.

There was a marked mortality in all concentrations of *S. subsimplex* extract (Figure 5). The lines

representing these treatments show a rapid decline. In addition, no progression in the number of dead caterpillars was seen in the control group during the time these individuals were observed. The observed results showed differences in the values, although not significant according to the Fisher's test.

Table 7: Number of live and dead caterpillars observed in the T1 and in the T2 or T4 after 72 hours of contact of second-instar fall armyworm with Sematophyllum subsimplex extract.

	Treat. 1 (2.00 µg/µl)	Treat. 2 or 4 (1.00 ou 0.25 µg/µl)	Total
Live	2	0	**2**
Dead	3	5	**8**
Total	**5**	**5**	**10**
p-value (Fisher)	**0.2222**		
p-value (Tocher)	**0.2222**		

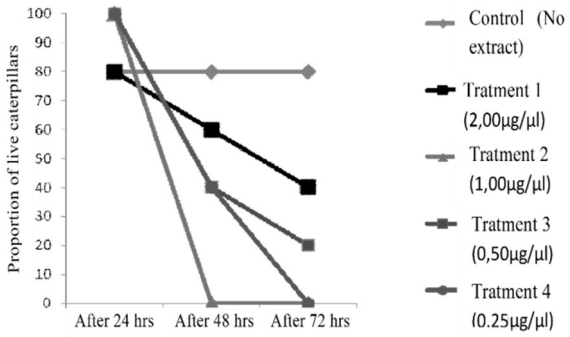

Figure 5: Evolution of the proportion of live Spodoptera frugiperda caterpillars under different extract concentrations of Sematophyllum subsimplex. Three-day assessment (24, 48 and 72) of contact of the insect with the treated food.

In relation to antifeedant activity, the present study showed that the application of 0.25 µg/µl of *S. subsimplex* extract is enough to control second-instar *S. frugiperda* caterpillars regardless of external factors. However, all concentrations caused phagodeterrence to the larvae, indicating the potential of the ethanolic extract of *S. subsimplex* in insect control, even at small concentrations. A similar result was obtained by Frahm et al. (2002) with the moss *Neckera crispa* Hedw. They tested the effect of the ethanolic extract at concentrations of 0.5%, 1.0% and 2.5% against the slug *Arion lusitanicus* (MABILLE, 1868) and found phagodeterent action in the first two concentrations.

Another important work was that of Markham et al. (2006) who evaluated the damage caused by second-instar *S. frugiperda* larvae on soybean leaves after application of protein extract (2.0 mg/mL) from six moss species. *Ceratodon purpureus* (Hedw.) Brid. showed the most promising results, promoting the lowest index of herbivory and giving evidence of the presence of insect resistance genes in bryophytes. Nevertheless, the results of the present study are in line with the literature in that low anti-herbivory effects were obtained as a consequence of low intake of food treated with different *S. subsimplex* extract concentrations by second-instar caterpillars in the first 24 hours of the experiment.

Research with extracts or artificial diets has also demonstrated the insecticidal potential of bryophyte metabolites on caterpillars. For example, Ande et al. (2010) found that aqueous solutions of the mosses *Calymperes afzelii* Sw. and *Bryum coronatum* Schwägr. showed toxic activity (80% and 77% of larval mortality, respectively) against maize stem borers, being better or just as good as Tricel, an inorganic insecticide. In another study, sesqui- and diterpenoids present in the metabolism of the liverwort *Porella*

chilensis (Lehm. & Lindenb.) Trevis. displayed larvicidal activity when incorporated to the diet of *S. frugiperda* larvae, reducing larval growth and causing severe changes in the epithelial cells of the midgut (CORZO et al., 2012). Moderate to high doses of protein extracts of bryophytes such as *Octoblepharum albidum* Hedw., *Fissidens asperifolius* Broth. & M. Fleisch., *B. argenteum* and *Marchantia linearis* Lehm & Lindenb induced high mortality rates on *Helicoverpa zea* (BODDIE, 1850) and *Spodoptera litura* F. 1775, in addition to adverse effects on the development of survivors (KRISHNAN et al., 2013).

The findings of this study support Rodriguez et al. (1996) argument that the insecticidal action of plants is more efficient in the larval stage because larvae are more sensitive to the chemical substances present in the pre-treated food ingested. According to Roel (2001), the extent of the effects of the extracts and the time of action of insecticidal substances depend on the dosage used. However, less intense but longer lasting effects are obtained with smaller dosages. In the case of the ethanolic extract of *S. subsimplex*, all concentrations tested, including the lowest ones, showed insecticidal activity, demonstrating that this species has promising bioactive metabolites against caterpillars.

In the case of bryophytes, the advantage of testing extracts as alternative insect control agents is, according to Ande et al. (2010), the possibility to discover products of easy application, availability, safety, and low cost, allowing producers to practice an effective control in a sustainable and profitable manner. The few works alternative control of different caterpillar species, including *S. frugiperda*, employing bryophyte extracts have been developed in foreign countries, demonstrating positive results (ASAKAWA, 1981; MARKHAM et al*.,* 2006; HAINES et al., 2009; ANDE et al., 2010; CORZO et al*.,* 2012; KRISHNAN et al., 2013; RAMIREZ et al., 2017). In Brazil, only Silva et al. (2011) reported the feeding habits of Lepidoptera larvae (Geometridae) and adult micro-snails (Charopidae) consuming bryophyte species in Atlantic Forest, but these authors did not directly address the antifeedant and/or toxic effects of these plants for the invertebrates.

CONCLUSIONS

The tested concentrations of ethanolic extracts from *S. subsimplex* in the natural diet of second-instar *S. frugiperda* larvae deterred their feeding activity and consequent development and ecdysis, leading to larval death. This demonstrated the presence of chemical defense compounds in the constitution of this plant species, confirming antifeedant and insecticidal activity against *S. frugiperda* caterpillars. Thus, we conclude that as low concentrations as 0.25 µg/µl of *S. subsimplex* extract are enough to control *S. frugiperda* for this concentration presented antifeedant and insecticidal effect, being therefore indicated for the population control of the fall armyworm and, possibly, other species of the genus *Spodoptera*.

This was the first scientific study to test the biological activities *S. subsimplex* extracts and the results may serve as a subsidy for new studies with other concentrations and analytical parameters such as larval and pupal phase duration, fecundity and longevity. In view of the biotechnological potential of the said moss extract, we suggest the continuation of the study of this moss aiming at isolating, quantifying and producing the derivatives responsible for the deterrent and insecticide effects found here, with a view to

presenting a product compatible with sustainability in the agricultural environment and market demands. This is, therefore, an advance for the Brazilian research of phytochemical constituents of bryophytes and their defensive properties against pest insects.

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