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Insecticidal and antifeedant activity of *Calymperes lonchophyllum* Schwägr. (Bryophyta) on the fall armyworm *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae)

Bryophytes are plants suitable for research on natural substances. Many studies have shown that they contain compounds with insecticidal and antifeedant activities. Such chemical activity is often tested through bioassays with caterpillars, especially *Spodoptera* Guenée larvae. The insecticidal activity by ingestion and/or antifeedant activity of the ethanolic extract of *Calymperes lonchophyllum* Schwägr. on 3rd-instar *Spodoptera frugiperda* JE Smith larvae was evaluated. Five treatments (2.0%, 1.0%, 0.5% and 0.25% µg/µl and control) with five replicates were tested, each replicate containing one larvae. Antifeedant effect and mortality were evaluated after 24, 48 and 72 hours of application of the plant extract. Antifeedant activity was observed after 48 hours of contact of the caterpillars with the treatments 1, 2 and 3. As for mortality, 72 hours of exposure to *C. lonchophyllum* extract at concentrations of 2.00 and 1.00 µg/µl were necessary to produce an insecticidal effect in *S. frugiperda*.

Keywords: Calymperaceae; Bryophytes Extract; Insecticide Plants; Herbivory; Corn Pest.

Atividade inseticida e fagodeterrente de *Calymperes lonchophyllum* Schwägr. (Bryophyta) sobre lagarta-do-cartucho do milho *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae)

As briófitas são plantas adequadas para pesquisas de substâncias naturais. Muitos estudos demonstraram que contêm compostos com atividades inseticidas e fagodeterrentes. Tal atividade química é frequentemente testada através de bioensaios com lagartas, especialmente larvas de *Spodoptera Guenée*. A atividade inseticida por ingestão e/ou atividade fagodeterrente do extrato etanólico de *Calymperes lonchophyllum* Schwägr. em larvas de 3º ínstar de *Spodoptera frugiperda* JE Smith foi avaliada. Foram testados cinco tratamentos (2,0%, 1,0%, 0,5% e 0,25% µg/µl e controle) com cinco repetições, cada repetição contendo uma larva. O efeito fagodeterrente e a mortalidade foram avaliados após 24, 48 e 72 horas da aplicação do extrato vegetal. A atividade fagodeterrente foi observada após 48 horas de contato das lagartas com os tratamentos 1, 2 e 3. Quanto à mortalidade, foram necessárias 72 horas de exposição ao extrato de *C. lonchophyllum* nas concentrações de 2,00 e 1,00 µg/µl para produzir efeito inseticida. em *S. frugiperda*.

Palavras-chave: Calymperaceae; Extrato de Briófitas; Plantas Inseticidas; Herbivoria; Praga do Milho.

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

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INTRODUCTION

Plants synthesize diversity of secondary metabolites which the main function is to protect plants against predators, microbes, and diseases (ZAYNAB et al., 2018). Over 2000 plant species are known to have insecticidal properties and have been used since ancient times (GHOSH et al., 2012). Natural plant products represent an alternative tool for pest control management, its eco-friendly, promote susteinable products, bio-degradable, and less hazardous to human health (MIRESMAILLI et al., 2014; NI et al., 2021). The effects of phytochemicals on pests may be driven by repellency, inhibition of oviposition and feeding, alterations in the hormonal system, developmental disorders, malformations, infertility, and mortality in the various life cycle stages (ROEL, 2001; SAITO et al., 1998).

Corn (*Zea mayz* L.) is one of the most important foods on the planet. It is, however, severely attacked by *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae), popularly known as fall armyworm (CRUZ et al., 1999; BUSATO et al., 2002). Thus, because this is an important pest of maize crops and because of the evidence of studies on insecticidal and/or antifeedant activity of bryophytes, which have demonstrated positive responses in the control of *Spodoptera* Guenée (HAINES et al., 2009; ANDE et al., 2010).

Generally, bryophytes are not attacked by insects, snails, slugs, and other arthropods (DAVIDSON et al., 1990; GLIME, 2017) because they present a wide variety of lipophilic terpenes, aromatic compounds, fatty acid derivatives, esters and many others (COMMISSO et al., 2021). Many of these components have typical aromas, pungency, and bitter taste, and display an extraordinary array of biological activities and medicinal properties (ALAM et al., 2015; SABOVLJEVIĆ et al., 2016). Many of the secondary compounds of bryophytes possess antimicrobial, antifungal, cytotoxic, antitumor, anti-allergenic, antifeedant, and insecticidal activities, among others (OZTURK et al., 2018; COMMISSO, 2021; WOLSKI et al., 2021).

In the search for natural insecticides derived from bryophytes species of the Brazilian Amazon, in this paper we present the results of a bio-directed study with *Calymperes lonchophyllum* Schwägr. (Calymperaceae), a moss often found on living or decomposing logs of native trees of the Amazon Forest. This species is widely spread in this region, and easily adaptated to different environmental conditions (MARTINS et al., 2014; COSTA et al., 2015; FAGUNDES et al., 2016).

Calymperes lonchophyllum extract has already been tested in the state of Pará, Brazil, for antibacterial effects (PINHEIRO et al., 1989). However, there are no studies on the potential of *C. lonchophyllum* phytochemicals to be used in the control of insect pests in agriculture (antifeedant/insecticidal activity), nor has this activity been reported to any other bryophytes in the country. *Calymperes afzelii* Sw., another species of the family Calymperaceae, has been evaluated for insecticidal activity against maize stem borer (*Elasmopalpus lignosellus* Zeller) (Lepidoptera: Pyralidae) by Ande et al. (2010), obtaining satisfactory results.

This work evaluated the insecticidal activity by ingestion and/or antifeedant effect of the ethanolic extract of *C. lonchophyllum* on 3rd-instar larvae of the fall armyworm *S. frugiperda*.

MATERIALS AND METHODS

Study Area

The study area was the Gunma Ecological Park (GEP), located at km 18 of the Augusto Meira Filho Highway (PA-391), in an Amazon Forest fragment, in the municipality of Santa Bárbara, northeast of the state of Pará, Eastern Brazilian Amazon (01°13'00.86''S and 48°17'41.18''W). It has an area of about 400 ha of native forest and 140 ha of open area for multiple uses, which has undergone several changes due to urban growth, hunting, and illegal logging (ALMEIDA et al., 2003).

The flora and ecosystems of the Park were studied under the project "Floristic Inventory and Phytosociological Analysis of the Environments of the Gunma Ecological Park, Municipality of Santa Bárbara, PA", whose results are published in Almeida et al. (2003). These authors established 20 permanent 1-ha plots, 15 of which were placed in non-flooded forest, two in igapó (nutrient-poor soils) flooded forest, and one in várzea (nutrient-rich soils) flooded forest, besides two plots placed in secondary forests in different successional stages.

The bryophytes of the park were studied by Fagundes et al. (2016), in their work on "Richness and ecological aspects of the bryophyte communities (Bryophyta and Marchantiophyta) occurring in a non-flooded forest fragment of the Gunma Ecological Park, Santa Bárbara Municipality, Pará, Brazil". The soils in the Park are classified into three types: Alic Yellow Latosol, Alic Concretionary Lateritic soil, and Low Humid Gley Soil (ALMEIDA et al., 2009). The climate type is Af_i - tropical humid, according to Köppen's classification (BASTOS et al., 1984), with a mean annual temperature of 26°C, minimum of 22°C and maximum of 31°C, annual rainfall varying from 2500 to 3000 mm, and relative humidity reaching ca. 85% (SUDAM, 1984).

Selection, collection, and screening of the botanical material

About 100g of fresh botanical material was collected in an Amazon Forest fragment at the Gunma Ecological Park, municipality of Santa Bárbara, Pará, Brazil (01°13'00.86'' S and 48°17'41.18'' W), in a non-flooded secondary forest ecosystem, on trunks of live trees, using the collection techniques of Yano (1989). The samples were washed, separated with the aid of tweezers (Flume 5) and a magnifying glass (Leica, Wild M3Z) and identified following the usual techniques for the group with the help of specialized literature (CRUM et al., 1981; FRAHM, 1991; LISBOA, 1993). The taxonomic classification of Goffinet et al. (2008) was adopted and the voucher samples were kept at the João Murça Pires Herbarium of the Emílio Goeldi Museum, in Belém, Pará, Brazil.

After the process of screening and identification of species, the botanical material was washed, air dried at an open sunny place and kept in a refrigerated environment (air-conditioned room). As these plants usually occur in clumps, mats, or tufts of individuals of different genera or species (RICHARDS, 1984), only specimens of *C. lonchophyllum* were separated, attempting to set apart the whole plant (gametophyte and sporophyte). This procedure was conducted at the Laboratory of Briology of the Emílio Goeldi Museum of Pará.

Collection of the ethanolic extract

The analyses were carried out at the Central Laboratory of Chemical Extraction of the Federal University of Pará (UFPA). A dry sample of *C. lonchophyllum* was weighed (6g) in a precision analytical balance (accuracy=0.01g) (Shimadzo, model AY220) and crushed in a pestle. This weight of dehydrated material is not standard, but it was sufficient to obtain the amount of extract desired for the experiment. The process consisted of three extractions, using the method of cold maceration (remaceration) at room temperature (25 to 30°C), with 95% ethanol (120ml), according to the usual methodology of the pharmaceutical industry.

Every two days the material was vacuum filtered and remacerated, renewing the extractive liquid only, for a period of six days to ensure that the largest number of substances were extracted from the plant matrix. The crude extract (final volume) of each step was placed in a volumetric flask, concentrated in a rotary evaporator apparatus (Büchi brand; model: Rotavapor R-3) under reduced pressure of 45°C ± 1°C (BARBOSA, 2004; SIMÕES et al., 2007). The extractive content was determined for the dry mass of 50 mg of *C. lonchophyllum*.

Obtaining stock solution and concentrations

For the experiments, the extract was diluted in Dimethyl sulfoxide (DMSO) to treat the *S. frugiperda* larvae. A stock solution was prepared using an extract mass/solvent volume ratio of 20mg/10ml. Dilutions (treatments) were prepared from this solution in the concentrations of 2.0; 1.0; 0.5 and 0.25 µg/µl (MARKHAM et al., 2006, adapted). For the control group, water diluted DMSO was used.

Breeding of *Spodoptera frugiperda*

Caterpillars were collected in a corn cultivation site near the Soil Sector of the Federal Rural University of Amazonia and from plants grown under greenhouse conditions. They were placed in transparent plastic gerboxes with dimensions of 12 x 12 x 3cm containing corn leaves (natural diet) to obtain the initial colony of larvae. The average duration of egg, larva, pupal and adult stage is around 3, 25, 11 and 12 days, respectively (GASSEM, 1996).

The caterpillars were grown in the Laboratory of Insecticide Bioecology (LABIN/ICA/UFRA), in an air-conditioned environment (24 ± 2°C) and relative humidity of 70 ± 10%. At the beginning of the pupal stage, they were transferred to new clean gerboxes with a moist filter paper at the bottom to avoid dehydration. The adults (moths) were put in PVC (polyvinyl chloride) tube cages of 30cm in diameter and 40cm in height, internally coated with filter paper for laying. The moths were fed with 10% honey solution, soaked in cotton pieces. The laid eggs were removed and packed in sterile gerboxes. The newly hatched larvae were transferred with a brush into cages made of PET bottles (MAIA et al., 2004), adapted for caterpillars. After the 1st-instar, the caterpillars were placed in disposable cups with artificial diet (BOWLING, 1967).

Experiment: instar selection and test

Twenty-five 3rd-instar, caterpillars between 10 and 12 days old were used in the bioassays. This instar was selected because, at the beginning of the larval phase, the caterpillars have a dietary preference for young leaves of the plant, and population growth can still be controlled (HARRISON, 1984; HOY et al., 1987). After larval development, the caterpillars infest the crop and burrow into the growing point (bud, whorl, etc.) of the plants, destroying their growth potential, or clipping the leaves, and sometimes burrow into the ear, causing severe damage to the crop (PARRA et al., 1995; GASSEN, 1996). Furthermore, it is important to consider that, in general, caterpillar's sensitivity to secondary compounds is higher in the early instars, decreasing as larval development progresses (BELLANDA et al., 2013). The larval viability of *S. frugiperda* was evaluated using different concentrations of crude ethanolic extracts of *C. lonchophyllum*, according to Borgorni et al. (2005), Markham et al. (2006) and Labbé et al. (2005), with adaptations.

Five treatments (2.0, 1.0, 0.5 and 0.25 µg/µl and the control with DMSO diluted in water) were applied. These concentrations were based on a work where the use of bryophyte extracts in the control of insects was tested, including the order Lepidoptera and the species *S. frugiperda* (MARKHAM et al., 2006; LABBÉ et al., 2005). The corn leaf sections used in the experiment were obtained from the cultivation of corn plots following the usual recommendations for fertilization and cultivation. Corn leaves were cut with a driller, obtaining disks of 2.3 cm in diameter. The amount of 50 µl of each treatment solution was pipetted into the leaf discs. The leaves were transferred to a 10 cm diameter Petri dishes previously lined with a moist filter paper disc; each Petri dishe received one caterpillar. The Petri dishes were kept moist throughout the experiment to preserve the turgescence of the leaf discs.

Experimental design

A completely randomized design with five treatments and five replications was used. Each replicate had one 3rd-instar larva (Figure 1).

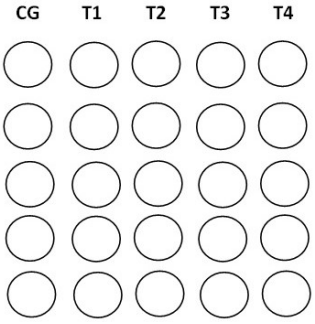

Figure 1: Scheme of the no-choice ingestion test with *S. frugiperda* treated with *C. lonchophyllum* extract. CG. Control Group; T1. Treatment with 2.0 µg/µl; T2. Treatment with 1.0 µg/µl; T3. Treatment with 0.5 µg/µl; T4. Treatment with 0.25 µg/µl

Data Analysis

Food consumption/antifeedant action and mortality of caterpillars were analyzed at 24, 48 and 72 hours after the onset of the experiment. For the first analysis, a scale of relative leaf damage was prepared

with scores varying from 0 to 5, adapted from Markham et al., 2006, as a quantitative comparison tool to determine the percentage of food consumption/antifeedant action in 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 measured by counting the number of dead caterpillars per day and these were removed from the plates after confirming the death.

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

As the number of dead caterpillars in the treatments was higher than in the control group, the Fisher test (SIEGEL, 1956) was applied, with confidence level $\alpha = 0.05$. In this case, the significance of the differences between the frequencies of two independent samples (control x each of the treatments) was verified. The samples in this design were small; thus, we used Tocher's Modification to verify the significance of not so extreme differences. Fisher's and Tocher's calculations represent the most powerful statistical proof for data arranged in 2 x 2 tables (SIEGEL, 1956). All analyses were performed in the software BioEstat 5.0 (AYRES et al., 2007).

RESULTS AND DISCUSSION

Antifeedant

24-hours assessment

Feeding scores were initially pooled to estimate food consumption, and Figure 2 shows the differences between the feeding behaviors of the different groups of caterpillars. The results of this experiment did not reveal major differences in the feeding behavior of the treatment groups in contrast with the control group.

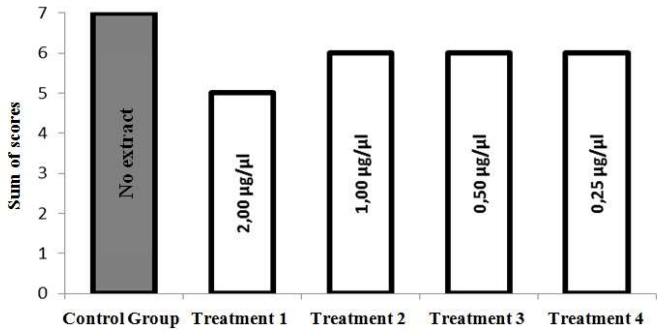

Figure 2: Sum of the consumption scores of 3rd-instar *S. frugiperda* 24 hours after contact with different concentrations of *C. lonchophyllum* extract.

The absence of differences in food consumption after 24 hours of onset of the experiment was confirmed by the U test, which showed no significant differences between the Control Group and Treatments 1 (p-value = 0.1481), 2, 3 and 4 (p-value = 0.3008). In this case, there is evidence that different concentrations

of *C. lonchophyllum* extract do not cause a significant antifeedant action in 3rd-instar fall armyworm larvae after 24 hours of contact with the food (Table 1).

Table 1: Mann-Whitney U tests for the effect of *C. lonchophyllum* extract on 3rd-instar fall armyworm larvae 24 hours after onset of food consumption, comparing the control group to each of the treatments.

	Control Group vs. T1 (2.00 µg/µl)	Control Group vs. T2 (1.00 µg/µl)	Control Group vs. T3 (0.50 µg/µl)	Control Group vs. T4 (0.25 µg/µl)
Z statistics	1.0445	0.5222	0.5222	0.5222
Critical value	1.6449	1.6449	1.6449	1.6449
p-value	0.1481	0.3008	0.3008	0.3008

The Mann-Whitney U test results for the effect of the different concentrations of *C. lonchophyllum* extract on the herbivory of 3rd-instar *S. frugiperda* larvae also showed no significant differences between any of the paired samples. Therefore, in this analysis, all extract concentrations had similarly efficient effect in terms of promoting antifeedant action.

48-hours assessment

Figure 3 shows that after 48 hours there were larger differences in the feeding behavior of caterpillars of the different groups. The results of the U test (Table 2) showed that the Control Group had a significantly higher effect than samples of each of the other treatments, namely, Treatment 1 (p-value = 0.0108), and Treatments 2 and 3 (p-value = 0.0473). This indicates that there was an antifeedant action induced by the respective concentrations (2.00 µg/µl, 1.00 µg/µl, and 0.50 µg/µl) of *C. lonchophyllum* extract when food consumption was measured after 48 hours of contact of caterpillars with the food.

Antifeedant action was also confirmed by Wada and Munakata (1971) in their assessment of the inhibitory activity of the pinguison sesquiterpene isolated from the liverwort *Aneura pinguis* (L.) Dumort. against *Spodoptera litoralis* Boisd. (Lepidoptera), testing for the effect of the concentrations of 0.5%, 0.25%, 0.125%, 0.63% and 0.031%, being the two largest (0.5%, 0.25%) concentrations similar to those used in the present study. Wada and Munakata (1971) found the best responses for food inhibition at the concentration of 0.5%. This confirms the antifeedant activity observed in the present study for the Treatment 3 (0.50 µg/µl).

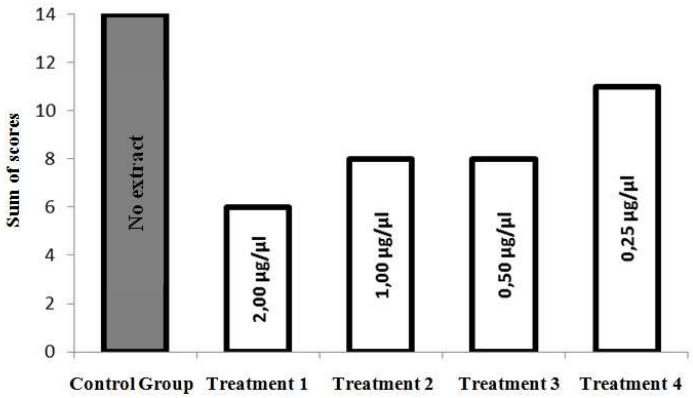

Figure 3: Sum of the consumption scores of 3rd-instar *S. frugiperda* 48 hours after contact with different concentrations of *C. lonchophyllum* extract.

Table 2: Mann-Whitney U tests for the effect of *C. lonchophyllum* extract on 3rd-instar *S. frugiperda* larvae 48 hours after onset of food consumption, comparing the control group to each of the treatments.

	Control Group vs. T1 (2.00 µg/µl)	Control Group vs. T2 (1.00 µg/µl)	Control Group vs. T3 (0.50 µg/µl)	Control Group vs. T4 (0.25 µg/µl)
Z statistics	2.2978	1.6711	1.6711	0.9400
Critical value	1.6449	1.6449	1.6449	1.6449
p-value	0.0108	0.0473	0.0473	0.1736

Regarding Treatment 4, corresponding to the lowest extract concentration (0.25 µg/µl), the U-Test did not indicate significant differences. However, Wada and Munakata (1971) found antifeedant action for this concentration (0.25%) of the extract of the liverwort *A. pinguis* against *S. litoralis*. Comparing the treatments with each other, the U test did not indicate differences between any of the treatments, either between treatments 1 and 2, 1 and 3, 1 and 4, 2 and 3, 2 and 4 or 3 and 4 (Table 3).

Table 3: Mann-Whitney U tests for comparison of herbivory of 3rd-instar *S. frugiperda* larvae under different concentrations of *C. lonchophyllum* extract. Pairwise comparisons between treatments performed after 48 hours.

	T1 (2.00 µg/µl) vs. T2 (1.00 µg/µl)	T1 (2.00 µg/µl) vs. T3 (0.50 µg/µl)	T1 (2.00 µg/µl) vs. T4 (0.25 µg/µl)	T2 (1.00 µg/µl) vs. T3 (0.50 µg/µl)	T2 (1.00 µg/µl) vs. T4 (0.25 µg/µl)	T3 (0.50 µg/µl) vs. T4 (0.25 µg/µl)
Z statistics	0.6267	0.6267	1.1489	0.0000	0.5222	0.5222
Critical value	1.6449	1.6449	1.6449	1.6449	1.6449	1.6449
p-value	0.2654	0.2654	0.1253	0.5000	0.3008	0.3008

72-hours assessment

Figure 4 shows the existence of differences in food consumption in the treatments when compared to the control. The U-test (Table 4) indicated significant lower scores in Treatments 1 and 2 (p-value = 0.0236 and 0.0473, respectively) in relation to the Control Group. This is evidence, therefore, that *C. lonchophyllum* extract at concentrations of 2.00 µg/µl and 1.00 µg/µl has antifeedant action on 3rd-instar corn caterpillars after 72 hours of contact with the food.

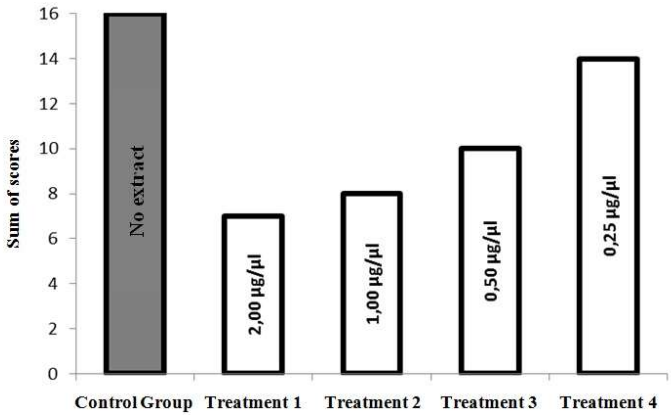

Figure 4: Sum of the consumption scores of 3rd-instar *S. frugiperda* larvae 72 hours after contact with different concentrations of *C. lonchophyllum* extract.

Regarding Treatments 3 and 4, the tests indicated that there were no significant differences (p-value = 0.0873 and 0.3008, respectively). This suggests that although feeding inhibition had already occurred 48 hours after the application of the extract, the group of caterpillars in this treatment resumed their feeding

activity. Thus, the hypothesis of antifeedant action of *C. lonchophyllum* extract at concentrations of 0.50 µg/µl and 0.25 µg/µl on 3rd-instar fall armyworm caterpillars after 72 hours of contact of the caterpillars with the food was rejected. The U-Test results of the pairwise comparisons between the different extract concentrations revealed no significant differences. These results are shown in Table 5.

Table 4: Mann-Whitney U tests for the effect of *C. lonchophyllum* extract on 3rd-instar fall armyworm larvae 72 hours after onset of food consumption, comparing the control group to each of the treatments.

	Control Group vs. T1 (2.00 µg/µl)	Control Group vs. T2 (1.00 µg/µl)	Control Group vs. T3 (0.50 µg/µl)	Control Group vs. T4 (0.25 µg/µl)
Z statistics	1.9845	1.6711	1.3578	0.5222
Critical value	1.6449	1.6449	1.6449	1.6449
p-value	0.0236	0.0473	0.0873	0.3008

Table 5: Mann-Whitney U tests for comparison of herbivory of 3rd-instar *S. frugiperda* larvae under different concentrations of *C. lonchophyllum* extract. Pairwise comparisons between treatments performed after 72 hours.

	T1 (2.00 µg/µl) vs. T2 (1.00 µg/µl)	T1 (2.00 µg/µl) vs. T3 (0.50 µg/µl)	T1 (2.00 µg/µl) vs. T4 (0.25 µg/µl)	T2 (1.00 µg/µl) vs. T3 (0.50 µg/µl)	T2 (1.00 µg/µl) vs. T4 (0.25 µg/µl)
Z statistics	0.2089	0.4178	1.4623	0.3133	1.2534
Critical value	1.6449	1.6449	1.6449	1.6449	1.6449
p-value	0.4173	0.3381	0.0718	0.3770	0.1050

Figure 5 shows the food consumption results of the caterpillar groups represented by the sum of scores at the three moments in which the experiment was performed, namely, 24, 48 and 72 hours after contact with the food, presenting an overview of the differences found. It was evident that food consumption decreased as the extract concentrations increased.

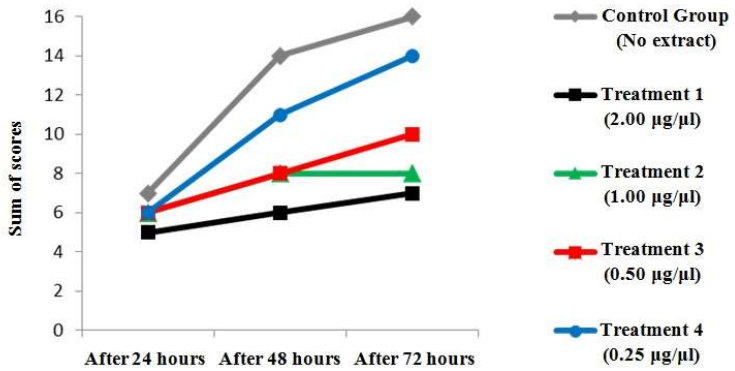

Figure 5: Evolution of the sum of consumption scores of 3rd-instar *S. frugiperda* larvae under different concentrations of *C. lonchophyllum* extract 24, 48 and 72 hours after contact with the extract.

Haines et al. (2009) analyzed the inhibitory action of mosses in before and after ingestion by the generalist caterpillar *Trichoplusia ni* Hu Hübner (Lepidoptera: Noctuidae: Plusiinae). The acceptability and quality of the mosses *Bryum argenteum* Hedw., *Climacium americanum* Brid., *Leucobryum glaucum* Hedw. and *Sphagnum warnstorfii* Russ. were compared with two control diets (lettuce and wheat germ) using this lepidonteron. Only control diets and the moss *C. americanum* were consumed, thus providing evidence of feeding inhibition in the case of most mosses. These results are in line with those found in the present study, confirming the antifeedant activity of mosses on lepidopteran larvae.

Mortality

24-hours assessment

Table 6 shows that after 24 hours of contact of the caterpillars with the treated leaves, deaths were observed only in Treatment 3. However, the number of deaths in the Control Group, in the group that received Treatment 3 and in the groups that received the other treatments, were not statistically different (p-value = 0.5000). That means that none of the concentrations of *C. lonchophullum* extract presented a significant insecticidal action on *S. frugiperda*. There were no differences between treatments.

Table 6: Number of live and dead 3rd-instar fall armyworm larvae observed in Treatment 3 and in the Control Group 24 hours after contact with *C. lonchophyllum* extract.

Control Group	T1 2.00 µg/µl	T2 1.00 µg/µl	T3 0.50 µg/µl	T4 0.25 µg/µl	**Total**
Live	5	5	4	5	**24**
Dead	0	0	1	0	**1**
Total	**5**	**5**	**5**	**5**	**25**
p-value (Fisher) = 0.5000					
p-value (Tocher) = 0.5000					

48-hours assessment

The only group with different results to those of the Control Group in relation to the number of dead caterpillars was that of the Treatment 1. In the data presented in Table 7, the Fisher test applied for these two samples showed that the number of deaths in the Control Group and in the Treatment 1 was statistically equal, with no differences in mortality (p-value [Tocher] = 0.0833). The Fisher test showed no differences between Treatment 1 and each of the other treatments. Thus, there was no significant insecticidal action of any of the concentrations of *C. lonchophyllum* extract after 48 hours of contact of the caterpillars with the food.

Table 7: Number of live and dead 3rd-instar fall armyworm larvae observed in Treatment 1 and in the Control Group 48 hours after contact with *C. lonchophyllum* extract.

	Control Group No extract	T1 2.00 µg/µl	T2 1.00 µg/µl	T3 0.50 µg/µl	T4 0.25 µg/µl	**Total**
Live	4	3	4	4	4	**19**
Dead	1	2	1	1	1	**6**
Total	**5**	**5**	**5**	**5**	**5**	**25**
p-value (Fisher) = 0.5000						
p-value (Tocher) = 0.0833						

72-hours assessment

Treatments 1, 2 and 4 presented different results from those of the Control Group. The results of the Fisher test presented in Table 8 refer to both the comparison between the Control Group and the Treatment 1 and the comparison between the Control Group and the Treatment 2, since these two treatments had the same number of live and dead caterpillars. It was observed that the number of deaths in the groups that received the Treatments 1 or 2 was statistically higher than the number in the Control Group (p-value = 0.0238). It was observed that after 72 hours of contact of the caterpillars with the food, the concentrations

of 2.00 and 1.00 µg/µl of *C. lonchophyllum* led to significant insecticidal action.

Table 8: Number of live and dead 3rd-instar fall armyworm larvae observed in Treatments 1 or 2 and in the Control Group 72 hours after contact with *C. lonchophyllum* extract.

	Control Group No extract	T1 or T2 2.00 or 1.00 µg/µl	**Total**
Live	4	2	**6**
Dead	1	3	**4**
Total	**5**	**5**	**10**
p-value (Fisher) = 0.2619			
p-value (Tocher) = 0.0238			

Differences between Treatment 4 and the Control Group were also tested. Mortality did not differ between Treatment 4 and the Control Group (p-value [Tocher] = 0.0833) (Table 9).

Table 9: Number of live and dead 3rd-instar fall armyworm larvae observed in Treatment 4 and in the Control Group 72 hours after contact with *C. lonchophyllum* extract.

	Control Group No extract	T4 0.25 µg/µl	**Total**
Live	4	3	**7**
Dead	1	2	**3**
Total	**5**	**5**	**10**
p-value (Fisher)	0.5000		
p-value (Tocher)	0.0833		

Treatments 1 and 2 also presented significant differences when compared to Treatment 3, with the same p-value (Tocher) = 0.0238 (Table 10), when previously compared with the Control Group. Regarding Treatment 4, the Fisher test was applied to compare it with Treatments 1 and 2 (Table 11) and no significant differences were observed (p-value [Tocher] = 0.1032), a fact that allows us to conclude that the number of deaths in the groups that received Treatments 1 or 2 was statistically equal to that of Treatment 4.

Although the extract concentration used in Treatment 3 was higher (0.50 µg/µl) than that in Treatment 4 (0.25 µg/µl), a greater number (two) of deaths was observed in the latter. However, the differences between these treatments were statistically non-significant.

Table 10: Number of live and dead 3rd-instar fall armyworm larvae observed in the Treatments 1 or 2 and Treatment 3, 72 hours after contact with *C. lonchophyllum* extract.

	T1 or T2 2.00 or 1.00 µg/µl	T3 0.50 µg/µl	**Total**
Live	2	4	**6**
Dead	3	1	**4**
Total	**5**	**5**	**10**
p-value (Fisher) = 0.2619			
p-value (Tocher) = 0.0238			

Table 11: Number of live and dead 3rd-instar fall armyworm larvae observed in the Treatments 1 or 2 and Treatment 4, 72 hours after contact with *C. lonchophyllum* extract.

	T1 or T2 2.00 or 1.00µg/µl	T4 0.25µg/µl	**Total**
Live	2	3	**5**
Dead	3	2	**5**
Total			
p-value (Fisher) = 0.5000			
p-value (Tocher) = 0.1032			

In a field experiment in Nigeria, Ande et al. (2010) found that *C. afzelii* extract promoted high insecticidal activity on maize stem borer (*Elasmopalpus lignosellus,* Zeller) (Lepidoptera: Pyralidae), causing a greater action when compared to the activity of three other moss species tested. The insecticidal action of this moss was as good as that of Tricel, the inorganic insecticide used in the control group.

Figure 6 shows the proportion of live caterpillars in the respective treatments at each live and dead count. The control group line starts at 100% after 24 hours. Then, after 48 and 72 hours, this value drops to 80%, overlapping the lines of Treatments 4 and 3, respectively, and showing stagnation in the control group. Treatments 1, 2 and 4 showed a progressive mortality, and treatments 2 and 4 had only 40% of the caterpillars alive after 72 hours.

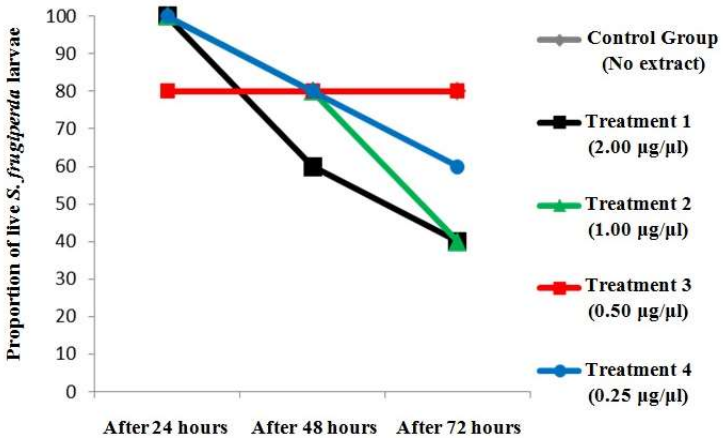

Figure 6: Evolution of the proportion of live *S. frugiperda* larvae under different concentrations of *C. lonchophyllum* extract. Observations after 24, 48 and 72 hours of contact of the insect with the treated food.

In general, higher mortality rates were observed among the higher concentrations of the extract (2.00 and 1.00 µg/µl), which confirms the hypothesis of this study that the higher the concentration of the extract, the greater should be the mortality.

CONCLUSION

Based on the statistical data, the results of the present study indicate *C. lonchophyllum* as a bryophyte species, with both insecticidal and antifeedant activity against 3rd-instar *S. frugiperda* larvae. This reinforces and confirms the existence of defense compounds present in the chemical constituents of this moss that may be of interest for the control of *S. frugiperda* occurring in corn plantations.

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