CONTROLE DA LAGARTA DO CARTUCHO (*SPODOPTERA FRUGIPERDA*) VIA INGESTÃODE DE SAMAMBAIA (*PTERIDIUM AQUILINUM*)

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RESUMO

Considerando a ação tóxica da samambaia de *P. aquilinum* para vertebrados, verificou-se sua atividade inseticida e uso potencial no controle de pragas agrícolas, testando seus efeitos sobre *S. frugiperda*. Extrato etanólico bruto foi feito com a parte aérea de *P. aquilinum*, sendo incorporado à dieta artificial (controle ou livre de extrato, 1% e 2%) com 50 repetições (lagartas). Houve mortalidade significativa (34% e 86%) nas concentrações de 1% e 2%, respectivamente, em comparação ao controle (10%). Nos tratamentos a 1% (20,03 dias) e 2% (20,42 dias), observou-se prolongamento do estágio larval (13,51 dias) no tratamento controle. Nos dias 3, 6, 9 e 12 após o tratamento, houve redução de peso, e o tratamento com 2% resultou em larvas 73% mais leves que o controle no dia 12. As pupas do tratamento a 2% foram mais leves que as demais. Em conclusão, 1% e 2% do extrato etanólico de *P. aquilinum* prolongaram o ciclo larval, diminuiu o peso das larvas e das pupas e, na concentração de 2%, causaram mortalidade efetiva de *S. frugiperda*.

Palavras-chave: bioinseticidas; manejo integrado de pragas; extratos vegetais; inseticidas botânicos; praga do milho.

FALL ARMYWORM CONTROL (*SPODOPTERA FRUGIPERDA*) THROUGH INGESTION OF BRACKEN (*PTERIDIUM AQUILINUM*)

ABSTRACT

Considering the toxic action of *P. aquilinum* bracken for vertebrates, its insecticidal activity and potential use in agricultural pest control was verified, testing its effects on *S. frugiperda*. Crude ethanolic extract was made with the aerial part of *P. aquilinum*, being incorporated into artificial diet (control or extract-free, 1% and 2%) with 50 replicates (caterpillars). There was significant mortality (34% and 86%) at concentrations of 1% and 2%, respectively, compared to control (10%). In treatments at 1% (20.03 days) and 2% (20.42 days), larval stage prolongation (13.51 days) was observed in the control treatment. On the 3rd, 6th, 9th and 12th days after treatment, there was weight reduction, and the 2% treatment resulted in larvae 73% lighter than the control did at day 12. The pupae of the 2% treatment were lighter than the others. In conclusion, 1% and 2% of *P. aquilinum* ethanolic extract extended the larval cycle, decreased the larvae's and pupae's weight, and, at a concentration of 2%, caused effective mortality of *S. frugiperda*.

Keywords: bioinsecticides; botanical insecticides; corn pests; integrated pest management; plant extracts.

INTRODUCTION

S. frugiperdae caterpillars are the main corn pest and widespread in Brazil and in the world, and, due to their polyphagous eating habit, can also be found in other crops such as soybean, cotton, wheat and oat (SILVA et al., 2017). As a consequence of these attacks, a great amount of chemical products have been used, causing environmental problems; for this reason, alternative management must be done in order to reduce the use of insecticides and the caterpillar population (FIGUEIREDO et al., 2006; LIMA et al., 2008). So that pest control happens coherently, the scientific community and agrochemical industries need to be constantly developing new tools for the control of insect

pests, with good control rates and small side effects (SPARKS, 2013).

The use of botanical insecticides presents itself as an interesting alternative, especially for organic systems, a market that has been growing worldwide (WILLER; LERNOUD 2016). Face the high demand for cleaner and safer products, extracts of vegetal origin have proved to be a very promising option for pest control, since they fit into the rules of programs developed by the Integrated Pest Management (IPM) and into norms of certifying companies on organic production (ISMAN et al., 2011; LIMA et al., 2008; ZANARDI et al., 2015).

Bracken (*P. aquilinum*) is an invasive species of pastures, forests, hillsides and found mainly in acid soils (MARÇAL, 2003). With worldwide distribution, it is found throughout Brazil, especially in the states of Rio Grande do Sul, Santa Catarina and Paraná, where it is related to intoxications caused in ruminants by ingestion of the plant (ANJOS et al., 2008).

P. aquilinum is described as an allelopathic, spontaneous plant (DOLLING et al., 1994; GLIESSMAN; MULLER 1978), and there are references to the efficiency of this plant's extracts on management of phytonematodes, lepidoptera and aphids (GERHARDT et al., 2012; NEVES et al., 2010; SELVARAJ et al., 2005).

Lovatto et al. (2016) using aqueous extracts of *P. aquilinum* and *Urtica dioica* on aphid *Brevicoryne brassicae* resulted in repellent and insecticidal action, offspring reduction and survival.

Considering the toxic activity of *P. aquilinum* reported in vertebrates and some insect pests, the objective was to verify whether this plant also has insecticidal activity on fall armyworm, assessing its potential for use in the control of this pest.

MATERIAL AND METHODS

The experiment was conducted at the Laboratory of Agricultural Entomology of University of Western São Paulo (UNOESTE) (Presidente Prudente/SP), under controlled conditions of temperature (26.0°C ± 1.0°C), relative humidity (60% ± 10 %) and photophase (12 h), with mass-farmed caterpillars, from March to April 2017.

For extract sourcing, completely-expanded *P. aquilinum* leaves were collected in rural area (Santo Anastácio-SP) and immediately taken to the laboratory. They were stored in Kraft paper bags and dried in air circulation oven at 60°C for 48 h. They were then ground (crushed) by a Willye-type grinder until a 0.45mm granulometry to obtain a fine powder that was put in hermetically-sealed glass containers and maintained at a temperature of 24°C without lighting until the extracts were manipulated.

The powder derived from the plant was macerated in 99ºethanol solution, with filtration once a week. Filtration was done through conventional glass funnel, using germination paper as filter. After filtration, the 99° ethanol was again poured into the container until covering 4 cm of the volume filled by the powder. This procedure was repeated until exhaustion to obtain the ethanolic extract as per methodology by Santana et al. (2013).

The solvent obtained was evaporated under reduced pressure in rotary evaporator (Quimis - Q344B), procedure used to obtain crude ethanolic extract. The extracted content was stored in a glass container protected from light and reserved to be added to the artificial diet (PARRA, 2001).

The amounts of 10 and 20 g of extract, respectively, corresponding to concentrations of 1.0% and 2.0% (P/V), were added to 1L of diet; the control treatment used 1L of diet without extract, thus composing the 3 treatments. The mixture was poured into gearbox-type containers, staying 40 minutes inside a laminar flow chamber with ultraviolet light for germicidal function; right after, it was stored in refrigerator until the inoculation of the caterpillars.

After preparation and cooling, the food was cut into cubes and standardized, each cube containing 4 grams on average. The cubes were individually put in 75mL plastic containers, where second-instar caterpillars were fed *ad libitum* artificial diet. Each treatment consisted of 50 replicates, each replicate being one caterpillar.

The caterpillars' mortality was daily observed until pupal stage. The caterpillars were weighed on the 3rd, 6th, 9th and 12th days after the start of the experiment (Precision Scale - Shimadzu® AUY 220). Later, after pupal formation, they were observed under binocular microscope for sex determination (BUTT; CANTU, 1962). After 24 hours of formation, the pupae were weighed and put in Petri dishes.

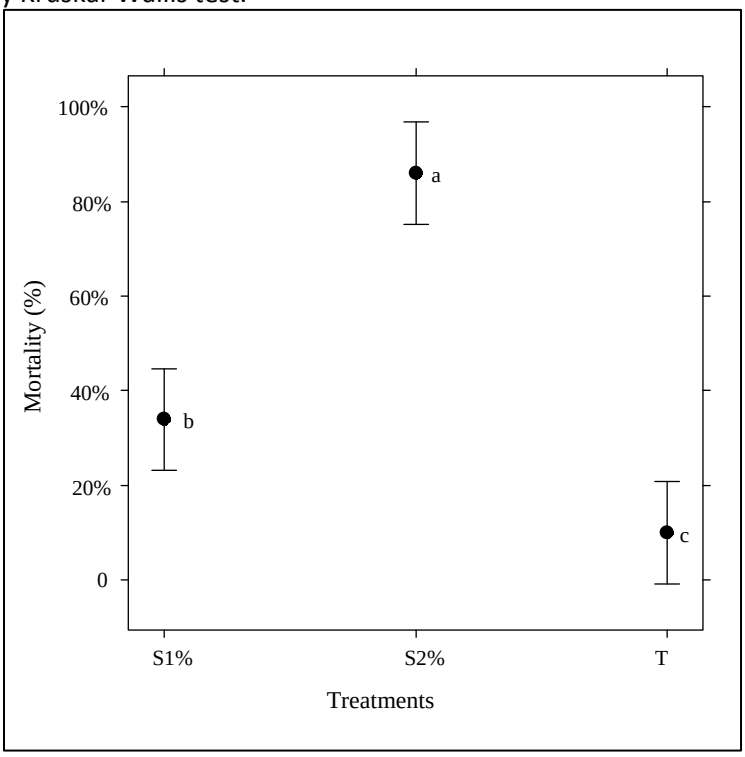
The experimental design used was of completely-randomized type with 3 treatments and 50 replicates. At the end of the assay, all assessed parameters were subjected to the

Shapiro-Wilk normality test; afterwards, the Kruskal-Wallis non-parametric test was performed on Action 3.0 (Estatcamp).

RESULTS AND DISCUSSION

At the end of the 24day period, it was possible to detect a significant mortality rate in samples containing concentrations of 1% of ethanolic extract (34%) and 2% of ethanolic extract (86%) of *P. aquilinum* compared to control (10%). (Figure 1). Selvaraj et al. (2005), assessing the effects of different *P. aquilinum* extracts on the biology of *Helicoverpa armigera* (Hübner, 1809) and *Spodoptera litura* (Fabricius, 1775) (Lepidoptera: Noctuidae), found up to 50% mortality for individuals exposed to the plant's chloroformic and ethanolic extracts. Observing the insecticidal activity of aqueous extracts of *P. aquilinum* (30% p/v), Gerhardt et al. 2012 also obtained positive responses on mortality of *Ascia monus teorseis* (Godart, 1819) (Lepidoptera: Pieridae) and *Myzuspersicae* (SULZER, 1776) (Hemiptera: Aphididae). In this case, the extracts resulted in mortality of 32% and 43% at 24h, and 60% and 63% at 48h of exposure to the treatments for *A. monuste orseis* and *M. persicae*, respectively, with these results being similar to those found with the commercial product OrganicNeem®.

Figure 1. Total mortality (%) at the 24th day after treatment of *S. frugiperda* caterpillars fed artificial diet containing *P. aquilinum* ethanolic extract. Legend: TT (extract-free), S1% (1% of extract), S2% (2% of extract). P. value> 0.05 by Kruskal-Wallis test.

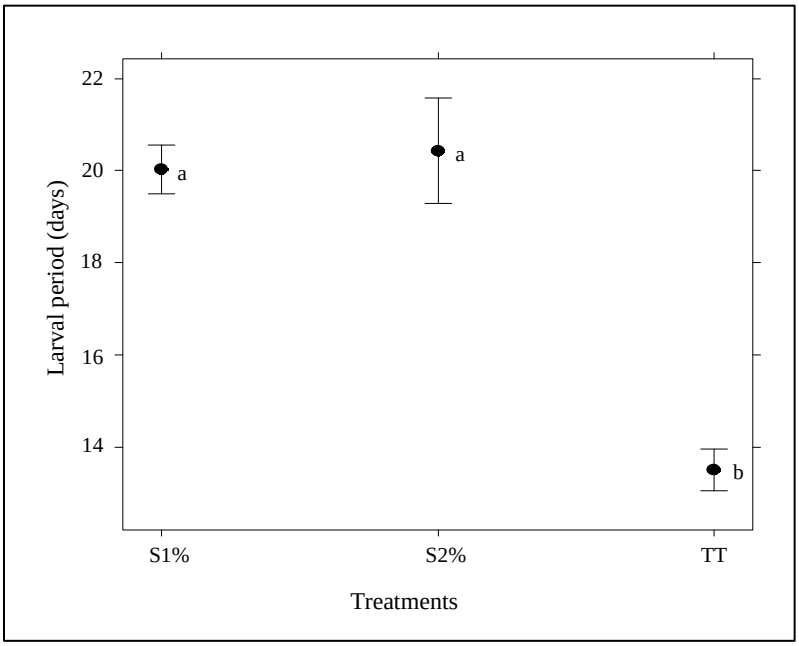


According to Costa (2009), the best known active principles of *P. aquilinum* include: quercetin, shikimic acid, prunasin, tannin, ptaquiloside, kaempferol and aquilide, which explains the plant's bioactivity on different organisms, as occurred in aphids and caterpillars exposed to the *P. aquilinum* extract described by Gerhardt et al.(2012), where there was a higher mortality in in the 48-hour exposure.

In treatments S1% (20.03 days) and S2% (20.42 days), there was significant prolongation of the larval stage of 7 days in relation to control (13.51 days) (Figure 2). This larval stage extension in the treatments may be related to several factors, including low food conversion, toxic substances or growth inhibitors (TORRES et al., 2001). This prolongation is not desirable because the caterpillars' cycle will be delayed and, consequently, the pest will be exposed for a longer time in the crop, eating normally, thus causing damages. However, there is controversy among authors; some claim that this prolongation of the larval stage within the crop will not affect the plant, since the insect will be vulnerable for a longer time to the attack of parasitoids, predators and entomopathogens

(fungi, viruses, bacteria, nematodes), emerged adults may be asynchronous with the normal population and, consequently, the copula could be hindered or, when it exists, would lead to consanguinity by the mating of same-generation individuals; others, in their turn, state that the insect staying longer in the crop will be raising expenses with chemical products to control these pests (RODRÍGUEZ; VENDRAMIM, 1996).

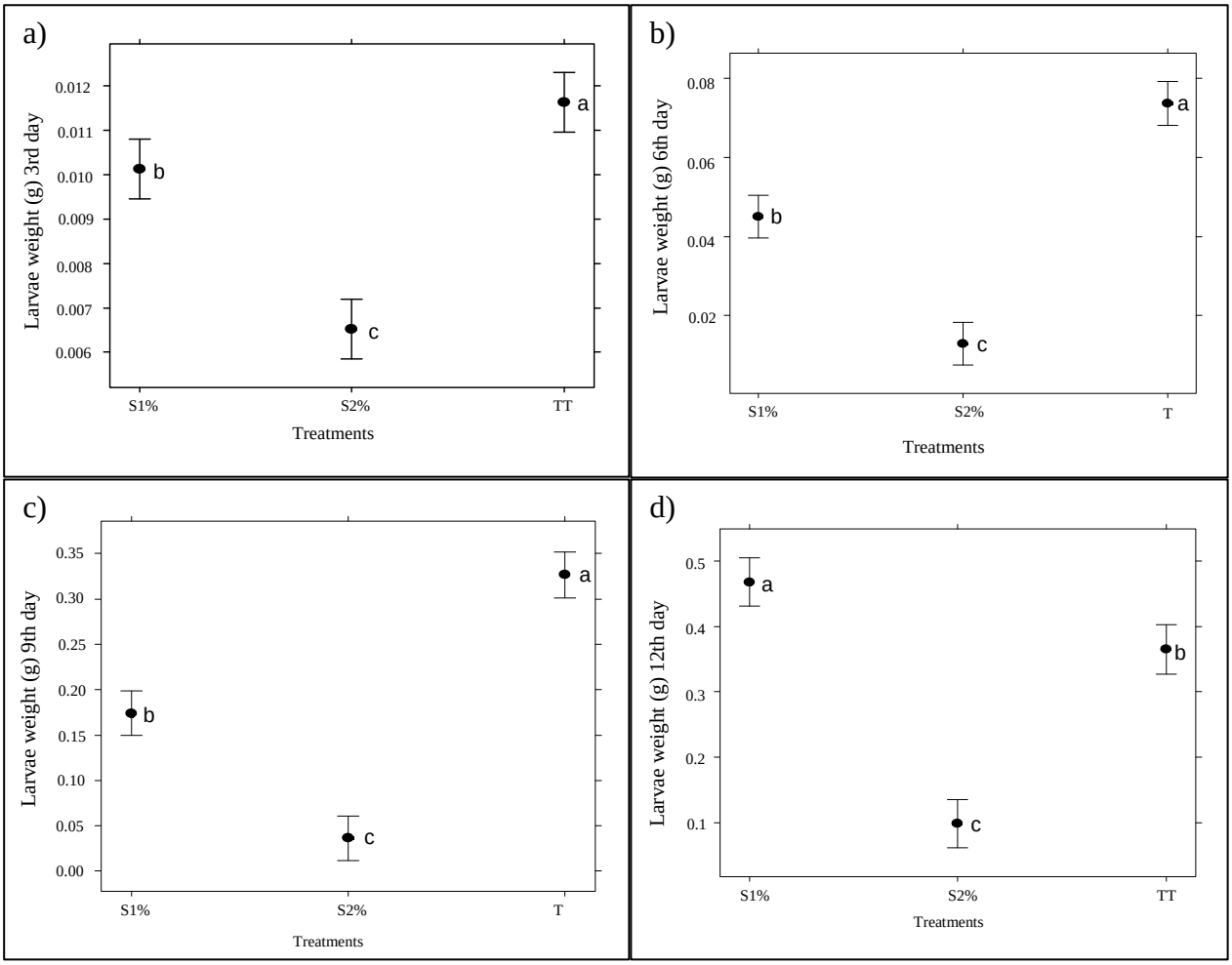
Figure 2. Cycle duration (days) of *S. frugiperda* caterpillars fed artificial diet containing *P. aquilinum* ethanolic extract. Legend: TT (extract-free), S1% (1% of extract), S2% (2% of extract). P. value> 0.05 by Kruskal-Wallis test.



According to Torres et al. (2001), the larval cycle is prolonged because the caterpillars do not absorb the nutrients necessary to complete their cycle, that is, the extracts have repellency molecules that prevent the caterpillars from eating normally, causing a delay until they have the quality and amount of nutrients required to complete the cycle. Inversely, using three concentrations (crude extract, 30% diluted extract and 10% diluted extract) of *P. aquilinum*, the longevity of insects *Brevicoryne brassicae* and *Myzuspersicae* reduced when compared to control, which lasted between 8 and 12 days more than treatments using extract (LOVATTO et al., 2013).

The caterpillars were weighed on the 3rd, 6th, 9th and 12th days for assessment of weight gain or loss in treatments using ethanolic extract (Figure 3).

Figure 3. Weight (g) of *S. frugiperda* caterpillars on the 3rd, 6th, 9th and 12th days, in artificial diet containing extracts of *P. aquilinum* leaves.



On the 3rd weighing day, there was difference between treatments; the control treatment was heavier (0.011 g) than the treatments containing plant extract (S1% - 0.010 g, S2% - 0.0065 g). On the 6th day, control individuals (0.073g) continued to have a greater weight gain, showing the extract's influence on the caterpillars' weight (S1% - 0.045 g and S2% - 0.012 g). Among treatments with extract there were differences as well, with caterpillars treated with extract at 2% being lighter than the others.

At the 9th weighing day, control individuals still presented weight gain (0.326 g), when, again, S2% (0.036 g) further reduced the caterpillars' weight compared to S1% (0.174 g). At the last weighing, on day 12, the control's mean weight was 0.280 g, S1% - 0.467 g and S2% - 0.098 g, showing that the extract affects the caterpillars' weight at both concentrations, with its reductive effect being greater at 2%.

Similarly, the methanolic extracts of seven plant species – *Centaurium erythraea*, *Peganum harmala*, *Ajugaiva*, *Aristolochia baetica*, *Pteridium aquilinume* and *Raphanus raphanistrum* – inhibited the growth of *Tribolium castaneum* larvae (JBILOU et al., 2008).

To Tanzubil and McCaffery (1990), this lack of weight gain shows that the insects, instead of using food during their growth, have their energies channeled to degrade possible secondary metabolites in the extracts. Neves et al. (2010), assessing *in vitro* the nematostatic activities of botanical extracts of *Meloidogyne javanica* and *M. incognita* (Ichinohe, 1952) (Tylenchida: Heteroderidae), found that aqueous extracts of *P. aquilinum* at 10% p/v reduced the

hatching of *M. incognita* and *M. javanica* in 90.4% and 80.7%, respectively.

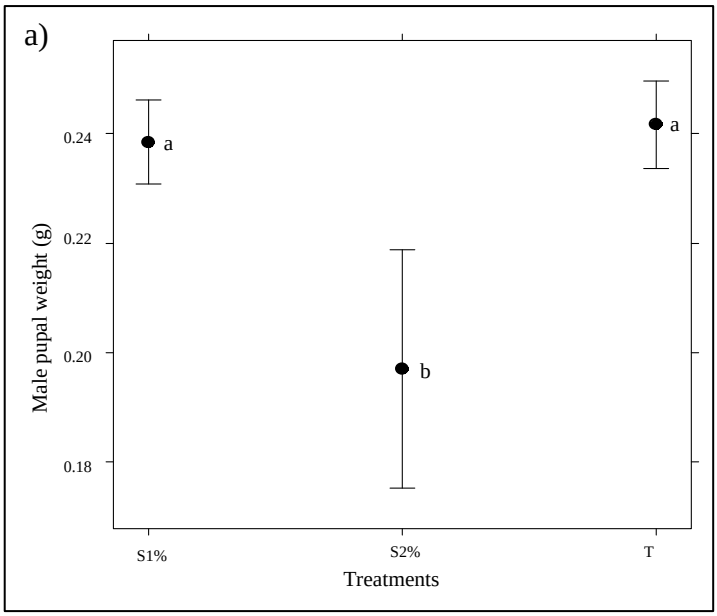
The weight loss in the control treatment on the 12th day is due to the fact that the caterpillars were in the beginning of the pre-pupal stage, and at this stage the caterpillar decreases in size and weight and interrupts the eating process for pupal formation, while the other treatments remained with the delayed cycle. Therefore, treatment S1% presented greater weight due to the prolongation of the larval phase, allowing the caterpillars to continue eating; in treatment S2%, the caterpillars were almost all dead.Assessing the pupae's weight, male and female groups were formed within the same treatments.

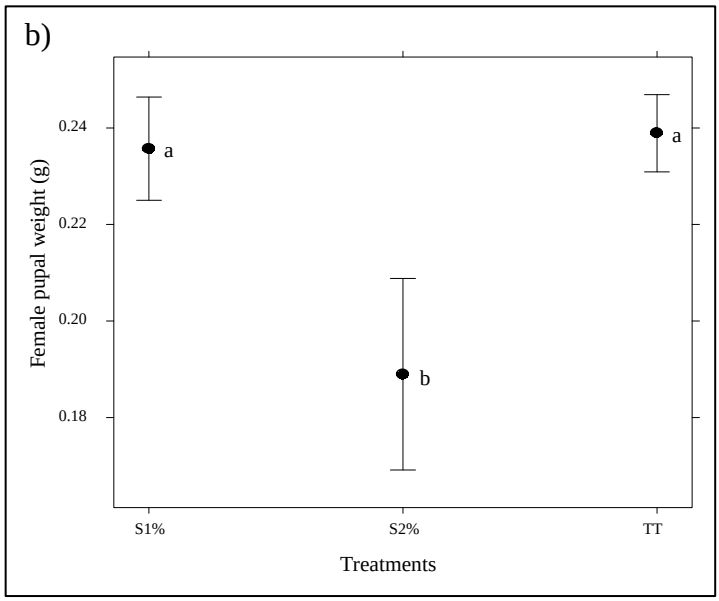
In the assessment of female pupae, there was difference between treatments. Control (0.238 g) presented similar results compared to S1% (0.235 g), making treatment S2% (0.189 g) different from the others. Among male pupae, there was no difference between control treatment (0.241 g) and treatment S1% (0.238 g), with S2% significantly reducing the pupae's weight (0.197 g) (Figure 4).

This reduction in the pupae's size in relation to control caused by *P. aquilinum* ethanolic extract has as consequence the fact that the pupae that reach the adult stage will give birth to small adults, with high probabilities of presenting copula problems.

In addition to the aforementioned studies referring to *P. aquilinum* formulations on pests of agricultural interest, the plant is recommended by Organic Horticulture manuals for management of insects and mites in vegetables (BURG; MAYER, 1999; HERTWING, 1986; SANTOS; SYLVESTRE, 2000; TIDEI et al., 1986), being mentioned by farmers of organic products from the southern region of Brazil as a viable and effective resource for this purpose (LOVATTO, 2012).

Figure 4. Weight of female and male *S. frugiperda*pupae fed *P. aquilinum*-based artificial diet. Legend: TT (control), S1% (1% of extract), S2% (2% of extract). P. value> 0.05 by Kruskal-Wallis test.





CONCLUSION

P. aquilinum extract has an ingestion action on *S. frugiperda* caterpillars, prolongs their larval cycle at 1% concentration and, at a concentration of 2%, and causes effective mortality of caterpillars.

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