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Effects of *Schinus terebinthifolius* extracts on the control of *Sitophilus* species in stored wheat grains

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The objective of this study is to evaluate the efficacy of *Schinus terebinthifolius* extract in controlling the insects of stored wheat. The experimental design was completely randomized with 10 replicates in a factorial scheme of 4 × 9, for extracts (control, aqueous extract, and hydro alcoholic extract of 5 and 15% of Brazilian pepper tree) and nine dates of evaluation (3, 6, 9, 16, 23, 30, 37, 44 and 51 days) after application. Ten weevil insects were inoculated into 200 g of wheat seed by applying 2 ml of each treatment. There is a control effect on the emergence and adults of *Sitophilus* species in wheat stored using *S. terebinthifolius* extracts. Up to the first nine days of storage, the hydro alcoholic extract (15%) has a relative efficacy of 35%. At 9 and 16 days of evaluation, hydro alcoholic extracts (5 and 15%) presented relative efficacy of 40%. At 44 and 51 days of evaluation, the aqueous extract shows relative efficacy of 40 to 60%. The aqueous extract appears to have an effect on the larvae and oviposition of the weevil observed by efficacy at 44 and 51 days. Hydro alcoholic extracts, on the other hand, show greater control over adults with efficacy until the 7th date.

Key words: *Triticum aestivum* L, storage, weevil, insecticidal action, Brazilian pepper.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most produced cereals for human consumption, because it is used for flour production and the manufacture of various food products (Scheuer et al., 2011). It is also an excellent source of food for animals, provided in the form of feed, hay, silage or pasture. In rural properties, its cultivation ensures economical flow and sustainability of production, being integrated into schemes of crop rotation in no-

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tillage system (Nunes et al., 2011).

After harvest, wheat grains can lose their quality quickly if not stored properly, especially due to insect infestation, fungal contamination and metabolic processes that reduce germination and seed vigor (Da Silva et al., 2013). One of the main insects attacking wheat grains is *Sitophilus* species (Coleoptera: Curculionidae), commonly known as weevil (Plarre, 2010).

Weevil is the main pest of stored grains; it has high biotic potential, cross-infestation, high penetration capacity in the masses of grain, a high number of hosts. Both adult insects as well as larvae are capable of damaging grains (Gallo et al., 2002). The damages result from the penetration of the larvae into the seeds; it causes reduction of weight, germination, nutritional value of seeds besides contaminating grains with uric acid, causing unpleasant odor and reducing the quality of flour (Lorini, 2003).

Chemical control is mostly used for the protection of stored grains from the infestation of pests due to its effectiveness, low cost and easy handling. However, many synthetic insecticides do not exterminate pests and prevent reinfestation, which can result in resistance of insects, presence of residues in food, possibility of operators' intoxication and environmental contamination (Lorini, 2003; Jardim et al., 2009). Bearing in mind the losses arising out of the indiscriminate use of these products, associated with consumers' concern about food quality, research and studies were motivated regarding new techniques for controlling these pests and, among these, is the use of plant origin insecticides (Santos et al., 2007a; Souza and Trovão, 2009).

The plant extracts have countless advantages compared to the use of synthetic products. Since they are obtained from renewable resources, they are rapidly degradable, act without leaving residues in food, do not damage the ecosystem and are less toxic to humans (Olivo et al., 2008; Agnolin et al., 2010). The Brazilian pepper tree (*Schinus terebinthifolius*) has been widely studied and its extracts fight against insect pests (Santos et al., 2007a, 2013). This is due to the chemical constituents distributed in plants' parts, such as tannin, bioflavonoids, triterpernic acids, mono and sesquiterpenes present in leaves (Silva et al., 2011). Considering the insecticide potential of the *S. terebinthifolius* leaves and seeking to promote alternatives to the use of synthetic insecticides in storage pest control, this study aims to evaluate the efficacy of extract of Brazilian Pepper tree in the control of weevils (*Sitophilus* spp.) in stored wheat.

MATERIALS AND METHODS

The experiment was carried out in the Laboratory of Entomology, Regional University of Northwestern region of the State of Rio Grande do Sul (Unijuí). The experimental design was completely randomized with 10 replicates in a factorial scheme of 4 × 9, for extracts (control, aqueous extract, and hydro alcoholic extract of 5 and 15% of Brazilian pepper tree) and nine dates of evaluation (3, 6, 9, 16, 23, 30, 37, 44, and 51 days) after application under controlled environment (temperature ± 25°C, relative humidity of 70 ± 10% and photoperiod of 12 h).

The wheat grains (clean and dry) used in the experiment were packed in plastic bags and kept in a freezer at a temperature of -10°C for seven days to eliminate any infestations of insects coming from the field. After this period in the freezer, the grains were transferred to glass vials and maintained in the laboratory at ambient temperature for 10 days with the aim of achieving hygroscopic equilibrium (Coitinho et al., 2011).

The insects of *Sitophilus* spp. used in the experiments were obtained from a mass of stored grains. Such insects were kept in glass containers of 5 L, sealed with plastic cover pierced and covered internally with nylon screen net to allow gas exchange. After the confinement for 15 days to perform fecundity, the insects were discarded. The containers were stored until the first generation appeared. This procedure was performed by successive generations, to ensure the quantity of adults necessary for the implementation of the experiments, using the methodology of Coitinho et al. (2011).

The *S. terebinthifolius* (Anacardiaceae) leaves were collected in the municipality of Ijui in November 2015 (spring), where minimum, average and maximum temperatures were around 15, 17, and 23°C, respectively. The collections were obtained from the specimens of this species from different locations of the tree canopy in the morning. The aqueous extract was obtained with the Brazilian pepper tree leaves through the process of infusion (Farmacopeia, 2010). The water was heated on a magnetic stirrer until it boiled. It was placed on the leaves using the proportion of 200 ml/68 g between solvent/solute (Santos et al., 2007b). The solution obtained was filtered and bioassays were immediately carried out. The hydro-alcoholic extract was prepared by maceration with ethanol 92% (v/v), using the proportion of 68 g/200 ml (Santos et al., 2007b) between solute/solvent. The solution obtained was filtered and the solvent was eliminated in rotating evaporator under reduced pressure, with controlled temperature of 60°C during the entire procedure, giving rise to the hydro alcoholic extract from the *S. terebinthifolius* leaves (Farmacopéia, 2010). The extract obtained was suspended again in distilled water to obtain concentrations of 5 and 15%.

The bioassays were carried out in plastic containers of 1000 mL, in which 200 g of wheat grains were placed. 10 specimens of *Sitophilus* spp. from 0 to 15 days of age were inoculated. With a pipette, 2 ml of aqueous extract and hydro alcoholic extract of 5 and 15% of Brazilian pepper tree were applied on the grains in 10 repetitions per treatment. This quantity of the extract allowed the whole grain mass to get moistened with the extracts. The containers were sealed with fabric to prevent air from entering. After the treatment, the experimental units were placed in a laboratory with temperature ± 25°C, relative humidity of 70 ± 10% and photoperiod of 12 h during the 51 days of completion of the experiment.

For the evaluation of the number of insects (dead or alive), counting was carried out every three days (counted from the application of the extracts) in the first nine days and subsequently every seven days for the 51 days of the study. All insects that moved any part of their body were considered alive, even those that remained immovable and only moved slowly when stimulated. Also, the relative effectiveness of treatments was quantified, estimated by the formula modified by Gonzalo et al. (2003): E% = (NITe - NITr / NITe) × 100; where E% is the effectiveness; NITe is the number of living insects of the control; NITr is the number of living insects of the treatment.

The data were subjected to analysis of variance using the program GENES (Cruz, 2013) and test for comparison of means was done by Tukey test at $p<0.05$ probability of error. This was followed with adjustment of regression equations as a function of

Table 1. Summary of analysis of variance for the treatments of extracts of Brazilian pepper tree in the control of *Sitophilus* spp. in stored wheat in different dates for evaluation.

Variation source	Degrees of freedom	Mean square (Alive insects)
Dates	8	5932*
Treatments	3	1426*
Treatments × dates	24	1064*
Error	324	66
Total	359	-
Mean	12.81	-
CV (%)	63.28	-

*Significant at 5% probability of error.

Table 2. Test of means for extracts of Brazilian pepper tree in the control of *Sitophilus* spp. in stored wheat in different dates for evaluation.

Treatment	Date (days)								
	3	6	9	16	23	30	37	44	51
Aqueous	9.9^{Aa}	9.5^{Aba}	9.2^{ABCa}	8.0^{Da}	7.0^{Da}	7.0^{Da}	7.0^{Da}	8.4^{BCb}	10.1^{Ad}
Hydro alcohol	9.4^{Ca}	9.1^{Ca}	7.4^{Da}	6.3^{Da}	6.3^{Da}	6.3^{Da}	6.4^{Da}	16.5^{Bab}	83.8^{Aa}
Hydro alcohol 15%	8.6^{Ca}	6.5^{Da}	6.5^{Da}	6.5^{Da}	6.7^{Da}	6.7^{Da}	5.8^{Da}	12.5^{Bab}	34.6^{Ac}
Control	10.0^{Ca}	10.0^{Ca}	9.9^{Ca}	9.9^{Ca}	9.7^{Ca}	9.7^{Ca}	9.6^{Ca}	19.8^{Ba}	50.5^{Ab}

*Averages followed by the same upper case letters on the line and lower case letters in the column do not differ statistically among themselves at 5% of error probability.

the date of assessment in each treatment tested.

RESULTS AND DISCUSSION

In the analysis of variance (Table 1), there was a significant effect of the assessment periods and extracts, as well as the presence of interaction. Through the averages test (Table 2), there was no difference between treatments until the 37th day of evaluation (7th date). At the 44th day of evaluation, the hydro alcoholic extracts (5 and 15%) decreased the viability of insects compared to the control ($p<0.05$). However, in this date, the aqueous extract was more effective in the control of the weevils, presenting a lower number of living insects compared to the other treatments. At the last date of assessment (51st), the aqueous extract was the most effective in controlling the weevils, followed by the hydro alcoholic extract (15%) of Brazilian pepper tree.

It was found that after the 37th day of evaluation, there was emergence of insects. This is possibly due to the fact that the life cycle of the weevil is 34 days (Gallo et al., 2002). In that date, the aqueous extract presents greater efficacy, indicating the effect of control over the larvae and eggs of the insect. This result is similar to that observed by Maroneze and Gallegos (2009), which also proved the best efficiency of the aqueous extract based on *Melia azedarach* leaves in the development of the immature and reproductive stages of *Spodoptera frugiperda.*

The insecticide effect promoted by *S. terebinthifolius* may be related to the chemical structure of compounds present in extracts from its leaves. Santos et al. (2013) identified the presence of phenolic substances, such as flavonoids from the aqueous extract and ethyl acetate from the leaves of *S. terebinthifolius*. Whereas Guimarães et al. (2014), evaluating the repellant, insecticide and fago inhibitor effect of the extracts of pepper dedo-de-moça (*Capsicum baccatum*) over corn *Sitophilus zeamais* weevil, found that the aqueous extract of seeds of pepper dedo-de-moça was the one that presented the greatest potential insecticide compared to the alcoholic extracts of 2.5, 5 and 10%. This corroborates the results observed in this study.

The aqueous extract has an inflection point of 45 days, showing that after the 5th day of evaluation, the extract begins to lose the effect in the control of weevils (Table 3). Considering the hydro alcoholic extracts, there is adjustment of equation of degree 3. In the first assessment dates, effect of the extracts was not observed with high population of insects. Over a period of time, the treatments increased their effectiveness by having a maximum control of the weevils, which was 30 and 37 days for the hydro alcoholic extract of 5 and 15%, respectively. From this period, there was no more effect of extracts, because the number of living insects begins

Table 3. Adjustment of the regression equation considering the different dates of evaluation of wheat grains for the treatments of extracts of Brazilian Pepper in the control of *Sitophilus* spp.

Treatment	Equation	R^2 (%)
Aqueous	$y = 12.30 - 0.18x + 0.0017x^2$	79.65
Hydro alcohol 5%	$y = -18.96 + 3.33x - 0.10x^2 + 0.0008x^3$	91.05
Hydro alcohol 15%	$y = -0.29 + 0.99x - 0.031x^2 + 0.0002x^3$	92.49
Control	ns	ns

R^2 = coefficient of determination; ns: Not significant.

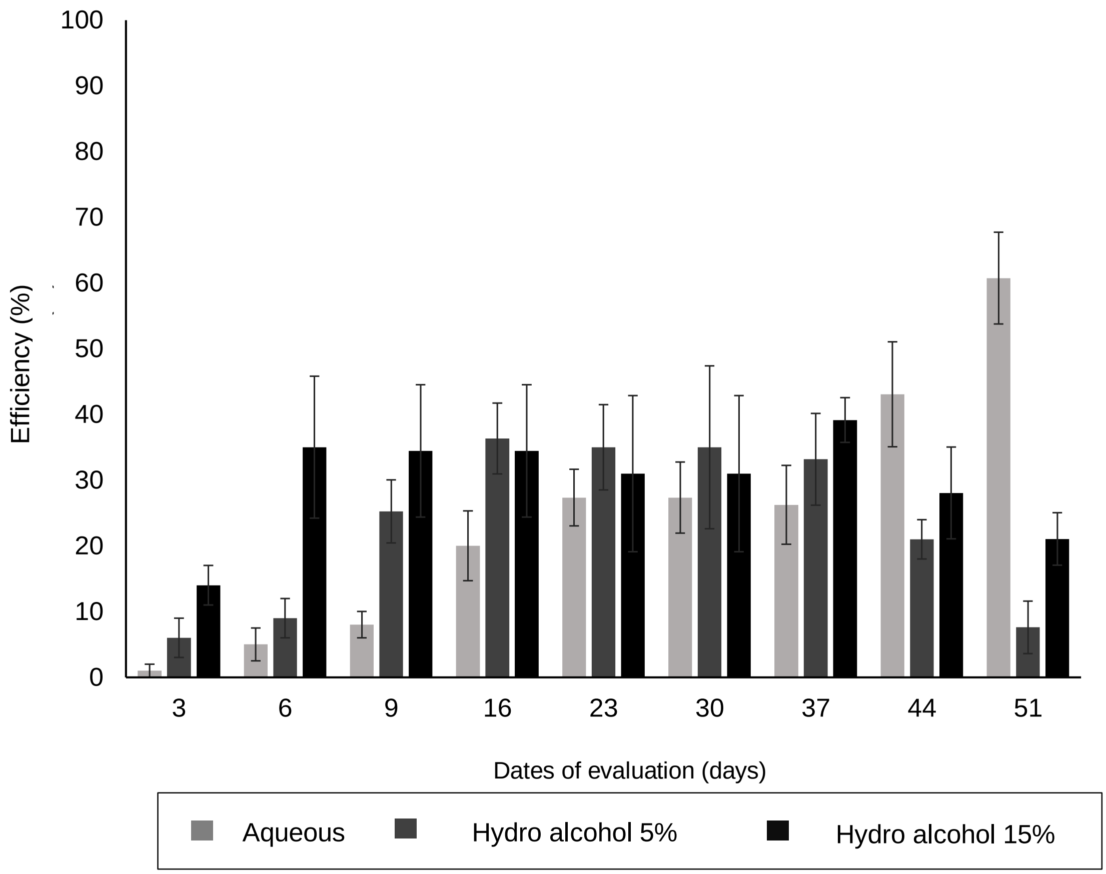

Figure 1. Relative efficiency (%) and standard deviations of the extracts of Brazilian pepper tree for the control of *Sitophilus* spp. in wheat grains.

to increase.

Regarding the relative effectiveness, the hydro alcoholic extract (15%) showed greater effect in the first nine days of storage, reaching 35% of effectiveness, in the initial period (Figure 1). After this date, the hydro alcoholic (5%) and aqueous extracts exhibited relative efficiency of 36 and 27%, respectively, in controlling the weevils; the effectiveness of the three excerpts remained very close until the 37th day of evaluation, when the life cycle of the weevil ends (Gallo et al., 2002). After the 37th day, there was emergence of new insects in the treatments period in which the aqueous extract stands out with relative efficiency of 61%. This indicates the effect of repellency, verified by the reduction or suppression of fecundation, in order to avoid reinfestation of insects in grain mass. For Coitinho et al. (2011), the smaller the infestation, the larger the repellency of the extract, a fact which favors the reduction of the fecundation and the number of insect’s hatching. For Gullan and Cranston (2008), repellency is a reaction of the sensory system of the insect, when it is exposed to undesirable substances. The insects have chemical receptors located in various parts of their bodies and are responsible for assessing the conditions of the environment where they are running away in unfavorable conditions.

According to Pinto Júnior et al. (1997), an insecticide can only be considered effective if it presents minimum efficacy of 80%. However, several authors observed the insecticide effect of the extracts, but with relative efficiency below this rate. This is also observed by Souza and Trovão (2009) by testing the efficiency of neem extract on the mortality of *S. zeamais* in stored maize. In the present study, the effectiveness occurred at levels below 80% in all treatments and the aqueous extract presented the highest value in the experiment, reaching 61% of maximum effectiveness. What demonstrates that effectiveness of 80% may not be adequate when one considers extracts of plants or species are not well known, as *Schinus*, which controlled the population of weevils with relative efficiency than the index analyzed. Estrela et al. (2006) concluded that the effectiveness of the oil from *Piper hispidinervum* and *Piper aduncum* leaves in *S. zeamais* depends on the path of intoxication and the concentration of the oil applied. For Regnault-Roger (1997), the toxic effect of the extracts and essential oils involves many factors, including the location of entry of toxins, since they can be inhaled, ingested or even absorbed by the tegument of insects.

The purpose of demand by plants with insecticide properties should not always be focused on the insect's mortality, because mortality is only one of the effects. The mortality requires a higher concentration of the product, which requires a greater amount of raw material, which is often impractical from an economic and sustainable point of view. The main goal of natural products is to reduce or prevent oviposition, feeding and reproduction of insects (Vendramim and Castiglioni, 2000). In addition, it is important to develop studies concerning the action of plant extracts on the reduction of motor activity of the insect, time of life, change in sexual behavior in males, impotence and a reduction in reproduction of pheromone, and if there is acute mortality in other insects, in addition to *Sitophilus* spp. (Almeida et al., 2005). The insecticides actions of these extracts should provide new studies to determine the chemical substances responsible for this effect and evaluate the effective concentrations in terms of the storage period, persistence, mechanisms of action and other aspects necessary to make commercial use of these plant extracts to control this pest.

Conclusion

There is an effect of control over the emergence and adults of *Sitophilus* spp. in stored wheat using *S. terebinthifolius* extracts.

Until the first nine days of storage, the hydro alcoholic extract (15%) shows relative efficiency of 35%. From the 3rd to 7th assessment date, the hydro alcoholic extracts (5 and 15%) present relative efficiency of 40%. At the 8h and 9th date of assessment, the aqueous extract presents relative efficiency from 40 to 60%.

The aqueous extract seems to have an effect on the larvae and oviposition of the *Sitophilus* spp. observed by effectiveness on the 8 and 9th date. Whereas the hydro alcoholic extracts show better control over the adults with effectiveness until the 7th date.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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