

Insecticidal effect of hydroalcoholic extracts of *Pleurotus ostreatus* against *Sitophilus zeamais*

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ABSTRACT

The maize weevil (*Sitophilus zeamais* Motschulsky, 1855; Coleoptera, Curculionidae) is one of the most important pests associated with stored grains. It is controlled almost exclusively with synthetic insecticides. Thus, leading to problems such as pollution and resistance development. Hence an alternative is the use of natural compounds such as edible mushrooms derivatives. The aim of this research was to assess, under laboratory conditions, the insecticidal and insectistatic effect of a hydroalcoholic extract of *Pleurotus ostreatus* (Jacq.) P. Kumm. 1871 against adults of *S. zeamais*. The parameters assessed were contact and fumigant toxicity, repellency, and antifeedant effect. The highest contact toxicity (57.5% mortality) was achieved with 30 mL extract L⁻¹ solvent. No treatment showed repellent effect; the repellency index (RI) values were higher than 1, and the antifeeding activity < 30%. The fumigant effect showed a maximum of 30% mortality with the highest concentration (300 µL L⁻¹ air). The hydroalcoholic extract of *P. ostreatus* exerts contact toxicity and interferes with insect reproduction or oviposition of *Sitophilus zeamais*.

Key words: Edible mushroom, maize weevil, oyster mushroom.

INTRODUCTION

One of the main difficulties in cereal production worldwide occurs in the post-harvest period, specifically during storage, due to significant damage caused by insect pests, rodents, fungi, and bacteria (Tefera et al., 2011). The presence of insect pests in stored grains results in the loss of cereal quality for both human consumption and seed use. Therefore, the conservation and protection of stored cereals is a food, social and economic necessity (Trivedi et al., 2018).

The maize weevil (*Sitophilus zeamais* Motschulsky, 1855; Coleoptera, Curculionidae) is a coleopteran widely distributed in the tropics and subtropics (Andrade et al., 2018). It is considered a key pest because the adults can bore directly into the kernels, with the rostrum females make holes in the grains to deposit eggs, and the larvae feed and transform into pupae and adults inside the seed. There is also a health risk associated with the consumption of weevil-infested grains, as damaged grain is prone to contamination by aflatoxins that are potentially carcinogenic substances (Nhamucho et al., 2017). According

to Santana et al. (2022), approximately 30% of cereal seeds may be infested by *S. zeamais* at the time of harvest. If the infestation continues in storage, about 30% to 50% of the grains are damaged within 6 mo.

Pyrethroids, and organophosphate insecticides, as well as phosphine fumigant have been used to control these species of insect (Souza et al., 2018). However, the indiscriminate use of these compounds has caused a series of problems such as environmental pollution, development of insecticide-resistant, and pesticide residues in the food (Santana et al., 2022). Today, the alternatives to synthetic insecticides are the use of vegetable-derived compounds such as edible mushrooms (Trivedi et al., 2018).

The use of microbial control agents, particularly entomopathogenic fungi, has been investigated to control a wide range of orchard and field crop pests. More than 750 species of fungi, mostly from hyphomycetes and entomophthorales are pathogenic to insects; many offer great pest management potential. Among the species already used in formulated mycoinsecticides are *Beauveria bassiana*, *B. brongniartii*, *Metarhizium anisopliae*, *Lecanicillium* spp., *Hirsutella thompsonii*, *Cladospodium oxysporium*, and *Isaria fumosorosea* (Maina et al., 2018). However, edible mushrooms have also been investigated as insecticides. For example, *Amanita muscaria* (L.) Lam. kills houseflies when mixed in a sugar solution, and *Trametes odorata* (Wulfen) Fr. powder keeps insects away from clothing. These observations suggest that some fungi may contain repellent, antifeedant, or even toxic compounds against insects. Rahman et al. (2011) indicated that *Pleurotus ostreatus* (Jacq.) P. Kumm. 1871 extracts obtained with solvents of different polarity such as petroleum ether, methanol-chloroform, and water have been effective for the control of *Tribolium castaneum* (Herbst, 1979). Likewise, these authors determined that the toxicity of *P. ostreatus* extracts and fractions increased with increasing exposure time. The fruiting bodies of some species of Basidiomycetes fungi possess insecticidal and nematocidal properties, making them a valuable source of new insecticides (Aguilar et al., 2017).

The insecticidal properties of these fungi are attributed to the presence of proteins such as lectins or hemolysins (Sivanandhan et al., 2018). Lectins are a type of protein present in plants, animals, and microorganisms. Several functions of them have been proposed, such as participation in the development of the fruiting body and the formation of mycorrhizae as a defense against predators and parasitoids (Trigueros et al., 2003). Regarding its mode of action, Jotic et al (2021) determined that the lectin Ostreolysin A6(OlyA6) produces pores in the midgut membranes of treated western corn rootworm (*Diabrotica virgifera* LeConte, 1868; Coleoptera: Chrysomelidae).

Pino et al (2019) assessed different solvents and obtained a fumigant effect against *Sitophilus zeamais* only with ethyl acetate. However, considering the high toxicity of this solvent, research is necessary to find alternative solvents of lesser toxicity capable of extracting many chemical compounds. Therefore, the aim of this research was to assess, under laboratory conditions, the insecticidal and insectistatic effect of a hydroalcoholic extract of *P. ostreatus* against adults of *S. zeamais*.

MATERIAL AND METHODS

Pleurotus ostreatus extract

Samples of *Pleurotus ostreatus* (Jacq.) P. Kumm. 1871 mushroom were obtained from an organic store in Chillán, Chile. During 24 h, at 40 ± 5 °C, 800 g body fruits were dehydrated in a forced convection oven (Memmert GmbH, UNB 500, Schwabach, Germany). Then, they were left at room temperature (20 ± 5 °C) in a container covered with absorbent paper for 7 d to eliminate moisture. The methodology to elaborate the extract is described by Pineda-Alegría et al. (2017). The dehydrated mushrooms were cut into pieces no larger than 2 cm, then placed in a container with 96% ethanol; then, distilled water was added in a ratio of 60:40 (ethanol:water). This solution was left to stand for 72 h. Once this time elapsed, it was filtered with a cloth to separate the larger particles and later with Whatman N°10 filter paper to later concentrate it in a rotary evaporator (Fisatom 802, Sao Paulo, Brazil) at a temperature of 90 °C and 40-45 rpm of rotation by 2 h, becoming the stock solution (100%).

Insects and cereal

Adults of *Sitophilus zeamais* Motschulsky, 1855, were obtained from permanent colonies of the Laboratory of Entomology of the Faculty of Agronomy, Universidad de Concepción, Campus Chillán, Chile. The population is maintained in a bioclimatic chamber (Memmert GmbH, IPS 749, Schwabach, Germany) under controlled conditions of 25 ± 2 °C, 60 ± 5 % relative

humidity, and total darkness, to obtain the F_1 individuals for the bioassays. The insect colonies were cleaned weekly by extracting all adults. The food substrate to colonies and bioassays consisted of maize (*Zea mays* L.) 'DK 440' (Dekalb, Anasac, Chile). It was acquired at the Experimental Station "El Nogal" of the Faculty of Agronomy of the Universidad de Concepción, Campus Chillán. To avoid any external insect contamination, maize was cleaned by removing harvest residues and then washed with tap water and refrigerated at 4.0 ± 5 °C for 1 wk.

Repellent effect bioassay

Repellency was assessed with the methodology of Mazzonetto and Vendramim (2003). A choice arena formed by five plastic Petri dishes of 5 cm in diameter and 1.5 cm in height was used, with a central dish connected to the other four by diagonally placed plastic tubes of 10 cm in length. In glass containers with 160 g maize, *P. ostreatus* extract solution was applied in the respective solvent (distilled water and ethanol at a ratio of 60:40) at 10, 50, and 100 mL extract L^{-1} solvent plus a control. Then, each treatment was placed in two diagonal opposed Petri dishes while the control, consisting of the grain mixed with the solvent, was placed in the other two dishes. Subsequently, 50 adult insects were released in the central plate without sex differentiation, and after 72 h, the number of insects in each plate was counted. Each treatment had 10 replicates, and Mazzonetto and Vendramim (2003) formula was used to calculate the repellency index (RI). The applied equation was $RI = 2G/(G + P)$, where G is the percentage of insects on the plant test, and P is the percentage of insects in the control. This index classifies treatment as neutral if $RI = 1$, attractive if $RI > 1$, and repellent if $RI < 1$.

Contact toxicity bioassay

Bioassays were conducted using the methodology of Obeng-Ofori and Reichmuth (1997). Glass containers of 500 mL were used, in which 100 g corn grains were mixed with 5 mL of a solution of *P. ostreatus* extract diluted in the solvent at concentrations of 10, 50, 100, 200, and 300 mL extract L^{-1} solvent, plus a control treated with the solvent (distilled water and 96% alcohol in a ratio of 60:40). The containers were shaken manually for 30 s to achieve homogeneous coverage of the grains by the solution and then left at room temperature for 20 min for solvent evaporation. Subsequently, each container was infested with 20 pairs of insects, which were differentiated by sex according to the criteria of Halstead (1963). Finally, the containers were covered with perforated lids to allow gas exchange and stored in a bioclimatic chamber at 26 ± 2 °C, $60 \pm 5\%$ RH, and total darkness. Each treatment was carried out with 10 replicates. The insect mortality was recorded at 24, 48, 72 h, and 7 d after infestation (DAI), quantifying the percentage mortality, which was corrected with Abbott's (1925) formula. At 55 DAI, insect emergence (F_1), weight loss, and grain germination were assessed. The percentage of adult insect emergence (F_1) was recorded, counting all adult insects that emerged after that period and considering as 100% the F_1 of control. Kernel weight loss was obtained by the weight difference between the initial (100 g) and final kernel weight at 55 DAI. In the germination test, we used 20 seeds (without apparent damage) per replicate. These seeds were placed for 7 d in Petri dishes conditioned with moist filter paper at 25 ± 5 °C in a bioclimatic chamber. The germination percentage was calculated considering as 100% the number of seeds per Petri dish.

Fumigant toxicity bioassay

The evaluation of fumigant toxicity was carried out using the methodology of Chu et al. (2011). A circular filter paper of 2.5 cm in diameter was treated with the undiluted *P. ostreatus* extract at volumes of 30, 60, 100, 150, 200, 250 and 300 μL extract L^{-1} air. The circular filter paper was placed to the inside face of the lid of a 150 mL container containing 20 g corn infested with 10 adult insects without sex differentiation. Each treatment was carried out with 10 replicates. The control was handled in similar way, but treated with the indicated solvent. The experimental units were stored at 25 ± 2 °C, $60 \pm 5\%$ RH, and total darkness in a bioclimatic chamber. Mortality was evaluated at 5 DAI, and the results were corrected with the formula of Abbott's (1925).

Antifeedant effect bioassay

The methodology of Rotundo et al. (2019) was used to evaluate the feeding activity. The hydroalcoholic extract of *P. ostreatus* was assessed at concentrations of 10, 50, and 100 mL extract L^{-1} solvent plus a control (solvent; ethanol 96% and distilled water in a ratio of 60:40). In a container, 100 mL distilled water was mixed with 40 g wheat flour and stirred during 20 min. Then, 1 mL was deposited on a non-stick silicone sheet and left to stand and dry for 5 d. Afterward, solid

discs were obtained, which were the food substrate for the insects in each bioassay. In transparent plastic Petri dishes with holes covered by a mesh for oxygen exchange, three disks were placed individually impregnated with 40 µL each concentration of hydroalcoholic extract of *P. ostreatus*. The control consisted of a Petri dish with three disks impregnated only with the solvent. Once the extract evaporated, the discs were weighed and placed in Petri dishes infested with five adults of *S. zeamais* without sex differentiation. After 15 d, the amount of food ingested by the insects on the treated discs were compared to the control. Each treatment had 10 replicates. The antifeedant activity was calculated using the antifeedant index (AI) of Farrar et al. (1989): $AI = [(C - T) \times C - 1] \times 100$, where C is consumption of food substrates in the control (mg) and T is consumption of treated food substrates (mg).

Experimental design and statistical analysis

The experimental design was completely randomized. Shapiro-Wilk and Levene tests were performed for testing the assumptions of normality and homogeneity of variances, and an ANOVA and a Tukey's test for comparison of means ($P \leq 0.05$) were carried out. Variables that did not meet the assumptions were subjected to a Kruskal Wallis nonparametric analysis with a significance of 5% ($\alpha = 0.05$). All analyses were performed with InfoStat software.

RESULTS AND DISCUSSION

Repellent effect

The results of repellency tests recorded RI values greater than 1, which according to Mazzoneto and Vendramim (2003) means no repellent effect on adults of *S. zeamais* (Table 1). Results do not agree with Pino et al. (2019), who assessed extracts of *P. ostreatus* in ethyl acetate, distilled water, methanol, and hexane at concentrations of 0.5%, 1.0%, 2.0%, and 4.0% against adults of *S. zeamais*. Specifically, the treatments with ethyl acetate at the concentration of 1.0% presented, according to the criteria of Bustos et al. (2017), a very high repellency, while the extract with distilled water showed weak repellency. However, in the treatments performed with methanol and hexane, RI values greater than 1 were recorded in all the bioassays assessed, so there was no repellency. Similarly, Novo et al. (1997) had similar results using plant extracts, concluding that ethanol does not extract compounds with a repellent effect.

Contact toxicity

The highest contact toxicity against *S. zeamais* was 57% mortality at 7 d of exposure with a concentration of 300 mL *P. ostreatus* hydroalcoholic extract L⁻¹ solvent. The treatments 10, 50, and 100 mL extract L⁻¹ solvent provided mortality lower 50% for the same period (Table 2). The 200 mL extract L⁻¹ solvent treatment resulted in 47.5% of dead insects, not differing significantly from the 300 mL extract L⁻¹ solvent. The LC₅₀ and LC₉₀ obtained were 22.7 mL extract L⁻¹ solvent and 244.3 mL extract L⁻¹ solvent, respectively, indicating that a significant increase in concentration was required to reach a 90% mortality (Table 3). These results agree with Rahman et al. (2011), who achieved high toxicity against adults of *T. castaneum* with methanol extracts of *P. ostreatus*, and mortality correlated at 30 h of exposure time.

The most significant decrease in F₁ was obtained with the highest concentration (300 mL extract L⁻¹ solvent), reaching only 2.4% of insects emerged compared to the control (100% emerged). The 100 and 200 mL extract L⁻¹ solvent concentrations also presented significant differences to the control with the emergence of 4.9% and 9.5%, respectively. These results indicate that, although the 100 and 200 mL extract L⁻¹ solvent concentrations did not exceed 50% mortality (33.7% and 47.5%, respectively), they provide an insectistatic effect that interferes with insect reproduction or oviposition. The percentage of adult emergence of the remaining treatments showed values fluctuating between 81% and 85%, being significantly higher than the rest of the bioassays (Table 2). The results of our research indicate higher effect than the one

Table 1. Repellency index (RI) of an hydroalcoholic extracts of *Pleurotus ostreatus* against adults of *Sitophilus zeamais*.

Treatment	24 h	48 h	72 h	96 h
mL extract L ⁻¹ solvent				
10	1.00	1.03	1.60	1.38
50	1.47	1.25	1.06	1.05
100	1.40	1.10	1.00	1.02

RI < 1 repellent, RI > 1 attractant, RI = 1 neutral (Mazzoneto and Vendramim, 2003).

Table 2. Mortality by contact toxicity and emergence of *Sitophilus zeamais*, and weight loss and germination of maize grains treated with an hydroalcoholic extract of *Pleurotus ostreatus*.

Treatments	Mortality ¹	Emergence ²	Weight loss ³	Germination ⁴
mL extract L ⁻¹ solvent	%	%	g	%
10	6.25a*	85.65b	13.08c	77.50a
50	16.25ab	81.29b	12.57c	83.75a
100	33.75bc	9.57a	9.01b	88.75a
200	47.50cd	4.99a	6.96b	88.75a
300	57.50d	2.48a	3.05a	97.50a
Control	-	-	-	83.00a*

¹Contact mortality after 7 d of *S. zeamais* adults treated with a hydroalcoholic extract of *P. ostreatus*. Tukey's test: Coefficient of variation (CV) = 26.02. *Percentage of mortality corrected with Abbott's formula (Abbott, 1925).

²Percentage of F₁ adult insect emergence at 55 d after infestation (DAI), considering as 100% F₁ obtained in the control. Tukey's test: CV = 26.37.

³Weight loss at 55 DAI of corn grains treated with a hydroalcoholic extract of *P. ostreatus* and infested with *S. zeamais*. Tukey's test: CV = 13.41.

⁴Percentage germination at 7 d of seeds treated with a hydroalcoholic extract of *P. ostreatus*. Tukey's test: CV = 13.42.

In all four variables, mean treatments with a common letter in a column are not significantly different (P > 0.05).

Table 3. Lethal concentration 50% (LC₅₀) and 90% (LC₉₀) for contact and fumigant toxicity against *Sitophilus zeamais* of an hydroalcoholic extracts of *Pleurotus ostreatus*.

	N ¹	b ± SE ²	LC ₅₀ ³ (LC _{95%})	LC ₉₀ ⁴ (LC _{95%})	Pr > X ²⁽⁵⁾
Contact	200	1.24 ± 0.14	22.7 ⁶ (17.7-31.7)	244.3 ⁶ (131.9-642.6)	< 0.001
Fumigant	100	1.38 ± 0.26	125.1 ⁷ (79.5-332.6)	1054 ⁷ (377.8-10,294)	< 0.001

¹Number of treated insects.

²Slope value ± standard error.

³Lethal concentration at 50% of effect with fiducial limits at 95% probability.

⁴Lethal concentration at 90% of effect with fiducial limits at 95% probability.

⁵Model fit to straight line.

⁶mL extract L⁻¹ solvent.

⁷µL extract L⁻¹ air.

documented by Pino et al. (2019), using *P. ostreatus* extract in ethyl acetate, which only reduced F₁ by slightly less than 50%. Furthermore, those results were not inversely related to contact toxicity. We infer that hydroalcoholic extracts of *P. ostreatus* present promising contact toxicity and a negative effect on the emergence (F₁) of *S. zeamais* after being treated at concentrations higher than 300 mL extract L⁻¹ solvent in an exposure period of 7 d.

The highest weight loss of maize treated with the hydroalcoholic extract of *P. ostreatus* reached values of 12.57 and 13.08 g using the lowest concentrations (10 and 50 mL extract L⁻¹ solvent), without significant differences with the control. Weight loss began to decrease significantly with the concentrations of 100 and 200 mL extract L⁻¹ solvent, with losses of 9.01 and 6.96 g, being the concentration of 300 mL extract L⁻¹ solvent that reached the most significant difference with the control, registering a weight loss of 3.05 g (Table 2). The results again coincide with the ethyl acetate extract evaluated by Pino et al. (2019), but it should be noted that, although both treatments report a weight loss close to 8.0 g, the hydroalcoholic extract is much less toxic to mammals than ethyl acetate (Abubakar et al., 2017), making it a better alternative. Concerning the germination of treated maize grains with the hydroalcoholic extract of *P. ostreatus*, nonsignificant differences were observed among the treatments. However, a slight increase in germination was observed as the concentration increased (Table 2). The lowest germination recorded was obtained with the concentration of 10 mL extract L⁻¹ solvent with 77.5%. The concentrations of 50, 100, and 200 mL extract L⁻¹ solvent exceeded 80% of germinated seeds, and the treatment using 300 mL extract L⁻¹ solvent was the one with the best results, reaching 97.5% germination. Our germination values are higher than those of Pino et al. (2019), who used extracts in ethyl acetate, hexane, and methanol, achieving germination values lower than 50%. Treatments higher than 100 mL extract L⁻¹ solvent of the hydroalcoholic extract of *P. ostreatus* do not affect maize germination due to the concentration of ethanol absorbed

by seeds. According to Onwimol et al. (2019), ethanol production in damaged or weak seeds is high compared with healthy ones. Kodde et al. (2012) indicated an inverse correlation between ethanol production and seed quality.

Fumigant effect

The toxicity by fumigant effect from extracts increased mortality. However, none of the treatments evaluated showed toxicity close to 100%. The highest toxicity was obtained with the concentration of 300 $\mu\text{L extract L}^{-1}$ air, with a mortality of 30% (Table 4). The LC_{50} and LC_{90} obtained were 125.1 and 1054 $\mu\text{L extract L}^{-1}$ air, respectively. The toxicity by inhalation was lower than the toxicity by contact (Table 3). The results coincide with those by Pino et al. (2019), using methanol, hexane, and distilled water as solvents, but being less toxic than ethyl acetate who reach 100% mortality.

Antifeedant effect

The maximum antifeeding effect of the hydroalcoholic extract of *P. ostreatus* was achieved with the 100 mL extract L^{-1} solvent concentration with an AI of 22.4%. In contrast, the 10 and 50 mL extract L^{-1} solvent treatments did not show feeding deterrence obtaining an AI of 4.01% and 14.27%, respectively. Significant differences were observed only between 10 and 100 mL extract L^{-1} solvent concentrations, the lowest and highest concentration assessed (Table 5). These results agree with Rincón et al. (2014), who evaluated the antifeeding activity of *Pernettya prostrata* (Cav.) DC., a plant with presence of lectins similar to *Pleurotus* fungi, against *T. castaneum* and *S. zeamais*. They used extracts (1000 and 500 ppm) and fractions (100 and 300 ppm) from fruits, leaves, and stems, obtaining low or no feeding deterrence in the two insect species studied. *Pleurotus ostreatus* at the evaluated concentrations presents reduced levels of feeding deterrence. However, a slight deterrence increase is shown, but without significant differences, as the concentration of the hydroalcoholic extract increases. A study by Belmonte et al. (2013) in which the feeding deterrent action of a foliar lectin extracted from *Myracrodruon urundeuva* M. Allemão was assessed against adults of *S. zeamais* at concentrations of 3, 15, 30, 45, 75, and 150 mg lectin g^{-1} wheat. They determined that ingestion of *M. urundeuva* did not produce significant mortality of insects, but a decrease in the relative feed consumption rate, showing a strong deterrent action with an AI between 61% (3 mg g^{-1}) and 91% (150 mg g^{-1}). Our results evidence the effect of the lectins that, according to Mier et al. (1996), are the ones that provide the insecticidal properties to *P. ostreatus*. Thus, we consider that the hydroalcoholic extract does not contain lectins or its activity is low because the temperature of extract concentration in rotary evaporator was 90 °C by 2 h. According to Ynalvez et al. (2011), the ideal incubation temperature is 30-70 °C. However, these same authors indicate that if lectins are subjected to a temperature of 100 °C for 3 hours, they are not denatured but significantly lose their activity. Additionally, He et al. (2014) indicated that a high temperature and short treatment time might eliminate the anti-nutritional functions of lectins.

Table 4. Fumigant effect of a hydroalcoholic extracts of *Pleurotus ostreatus* against adults of *Sitophilus zeamais*.

Concentration	Mortality
$\mu\text{L extract L}^{-1}$ air	%
30	7.5a
60	7.5a
100	10.0ab
150	12.5ab
200	20.0abc
250	25.0bc
300	30.0c

Means with a common letter are not significantly different according to Kruskal Wallis test ($P > 0.05$).

Table 5. Antifeedant index (AI) of hydroalcoholic extracts of *Pleurotus ostreatus* against adults of *Sitophilus zeamais*.

Treatments	Antifeedant index (AI)
mL extract L^{-1} solvent	%
10	4.01a
50	14.27ab
100	22.44b

Means with a common letter are not significantly different according to Tukey's test ($P > 0.05$).

The hydroalcoholic extracts of *P. ostreatus* are promising in terms of contact toxicity against *S. zeamais* at the concentration of 200 mL extract L⁻¹ solvent. Since oyster mushrooms have an attractive commercial value as gourmet products, one alternative could be using their production residues as a source of insecticides. These residues have a lower concentration of active compounds than the commercial mushroom, but they may have an important role in combatting insect pests. Hence further studies should consider the possibility to evaluate residues and the use of the hydroalcoholic extract as a complement with another compound of plant origin to achieve potentiation effect.

CONCLUSIONS

The hydroalcoholic extract of *Pleurotus ostreatus* shows contact insecticidal activity and interferes with insect reproduction or oviposition of *Sitophilus zeamais*, but lacks significative fumigant, repellent, and anti-feeding effect.

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