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Original Article

In vitro evaluation of methanol extracts of edible fungi *Pleurotus ostreatus* and *Lentinula edodes* against *Rhyssomatus nigerrimus* Fahraeus

Avaliação *in vitro* de extratos metanólicos dos fungos comestíveis *Pleurotus ostreatus* e *Lentinula edodes* contra *Rhyssomatus nigerrimus* Fahraeus

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Abstract

The study aimed to evaluate the insecticidal activity of extracts of edible mushrooms *Pleurotus ostreatus* and *Lentinula edodes* against *Rhyssomatus nigerrimus.* Methanol:water extracts (70:30) of *P. ostreatus* and *L. edodes* were made and evaluated in two *in vitro* tests (exposure and immersion toxic effect) against *R. nigerrimus.* Subsequently, the toxicity test of the extracts against *Artemia salina* was evaluated. These extracts were subjected to colorimetric tests and gas chromatography-mass spectrometry. The results showed a mortality effect against *R. nigerrimus* of 50% for the *P. ostreatus* 2 extracts at a concentration of 20% in the immersion test. Likewise, in the toxic effect test, 90% mortality was observed after five days of exposure to a concentration of 10%. On the other hand, for the toxicity test, the extract that showed the values with the highest mortality against *A. salina* was *P. ostreatus*, starting with 80% mortality at 100µg/mL. The functional groups present in the extracts were saponins, coumarins, and alkaloids. Furthermore, the presence of more than 7 compounds in the mushroom extracts evaluated is reported. This study demonstrates the insecticidal activity of *P. ostreatus* and *L. edodes* fungal extracts and indicates the importance of using different *in vitro* tests to elucidate the mechanism of action for future studies.

Keywords: edible fungi, insecticidal activity, *Pleurotus*, agriculture.

Resumo

O estudo teve como objetivo avaliar a atividade inseticida dos extratos dos cogumelos comestíveis *Pleurotus ostreatus* e *Lentinula edodes* contra *Rhyssomatus nigerrimus*. Extratos metanol:água (70:30) de *P. ostreatus* e *L. edodes* foram elaborados e avaliados em dois testes *in vitro* (efeito tóxico de exposição e imersão) contra *R. nigerrimus*. Posteriormente foi avaliado o teste de toxicidade dos extratos frente à *Artemia salina*. Esses extratos foram submetidos a testes colorimétricos e cromatografia gasosa-espectrometria de massas. Os resultados mostraram efeito de mortalidade contra *R. nigerrimus* de 50% para o extrato de *P. ostreatus* 2 na concentração de 20% no teste de imersão. Da mesma forma, no teste de efeito tóxico, foi observada mortalidade de 90% após 5 dias de exposição à concentração de 10%. Por outro lado, para o teste de toxicidade, o extrato que apresentou os valores com maior mortalidade contra *A. salina* foi *P. ostreatus*, iniciando com 80% de mortalidade a 100µg/mL. Os grupos funcionais presentes nos extratos foram saponinas, cumarinas e alcaloides. Além disso, é relatada a presença de mais de 7 compostos nos extratos de cogumelos avaliados. Este estudo demonstra a atividade inseticida dos extratos fúngicos de *P. ostreatus* e *L. edodes* e indica a importância da utilização de diferentes testes in vitro para elucidar o mecanismo de ação para estudos futuros.

Palavras-chave: fungos comestíveis, atividade inseticida, *Pleurotus*, agricultura.

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1. Introduction

The biological activity of edible mushroom, especially *Pleurotus* spp. and their derivatives, have been explored as alternative methods for agricultural pests (Cedillo, 2016; Pineda-Alegría et al., 2017; Cruz-Arévalo et al., 2020; Comans-Pérez et al., 2021). In this search, it has been observed that the excessive use of chemical compounds has led to resistance of some pathogenic organisms (nematodes, bacteria and mites), contamination of the environment and the presence of hazardous residues in food for human consumption (RAPAM, 2017; Aguilar-Marcelino et al., 2023). Therefore, the use of these insecticides is being restricted, internationally and nationally through the Stockholm Conventions on Persistent Organic Pollutants and the Rotterdam Convention (SEMARNAT, 2015).

In addition, the search for biological material with insecticidal activity has shown difficulty on the adult stages of insects. Unfortunately, the adult stage is one of the most visible indicators of an infestation in the agricultural crop and where crop damage is sought to be stopped. Although there are reports of insecticidal activity in larval stages, these stages are not as visible to the naked eye in the crop because some occur below the soil of the affected area (Dively et al., 2020). For example, the soybean weevil, *Rhyssomatus nigerrimus* Fahraeus (Coleoptera: Curculionidae), is currently considered the most economically important pest due to its direct and indirect damage to soybean (López-Guillén et al., 2012). Currently, *R. nigerrimus* is controlled by insecticides such as organophosphates, pyrethroids and fipronil (currently restricted in Europe) and aluminium phosphide (RAPAM, 2017; Terán-Vargas and López-Guillén, 2013). However, due to the side effects that chemical insecticides have shown, different alternative control methods are being sought.

A review work was recently carried out where around 150 species and 90 species of edible mushrooms with insecticidal effects (*Drosophila melanogaster*, *Spodoptera littoralis*, *Tribolium* spp., *Diatraea magnifactella* and *Sitophilus zeamais*) were reported between 1876 and 2021 (Castañeda-Ramírez et al., 2022a). However, studies have not covered different strains of established fungi and have not been evaluated with the adult stage of *R. nigerrimus*. For this reason, the present study aimed to evaluate fungal extracts of the genus *Pleurotus* against *R. nigerrimus* soybean insects.

2. Material and Methods

The present study was carried out at the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Campo Experimental Rosario Izapa, Tuxtla Chico, Chiapas, Mexico.

2.1. Biological material

The insects were obtained from soybean crops in the municipality of Tapachula, Chiapas, Mexico (located at 14° 56" and 92° 10"). Adults of the weevil, *R. nigerrimus*, were collected by hand and stored in 1 L plastic jars covered with organdy cloth. The insects were fed with soybean pods in the transfer to the laboratory, and in the laboratory, they were fed with purple sweet potato "*Ipomea batatas*" (López-Guillén et al., 2016).

2.2. Edible mushroom extracts

The basidiomes (1 kg) were obtained from *Pleurotus ostreatus* mushrooms (nutri-hongos company, Cuernavaca Morelos) and *Lentinula edodes* (Edible mushroom laboratory of the Colegio de la Frontera Sur - ECOSUR). The basidiomes were dehydrated to a weight of 200 g, then cut into small pieces, respectively. The edible mushroom sample was placed in a methanol:water (70:30) solution and allowed to macerate for 24 h. A second extraction of compounds was carried out on the same material. In the second extraction, a methanol:water solution (70:30) was also used and was identified as the second maceration. The extracts were filtered through a funnel and 15 cm gauze, cotton, and coarse-pore filter paper (#50), and the samples were subsequently concentrated under reduced pressure in a rotary evaporator. Finally, the extracts were dried in a lyophilizer (labconco®) and stored refrigerated at 11 to 4 °C until use. For each species of fungus, two solutions were obtained, called first maceration and second maceration, which were called *P. ostreatus* 1, *P. ostreatus* 2, *L. edodes* 1 and *L. edodes* 2, respectively.

2.3. In vitro assays against R. nigerrimus

Immersion test: The insects were immersed for 20 seconds in the treatments at a concentration of 2.5, 5, 7.5, 10 and 20% of the four extracts obtained (*P. ostreatus 1, P. ostreatus 2, L. edodes 1* and *L. edodes 2*) and their respective negative control (distilled water) and positive control Fipronil insecticide (0.1%). The insects were exposed to the extract treatments and placed in plastic containers (250 mL), and mortality was observed every day for 15 days (Castañeda-Ramírez et al., 2022b).

Toxic effect test by exposure: 300 µL of extracts of *P. ostreatus* 1, *P. ostreatus* 2, *L. edodes* 1 and *L. edodes* 2 at concentrations of 2.5, 5.0, 7.5 and 10% were placed in Petri dishes (9 cm diameter x 1.5 cm height), respectively, sterile water was used as a negative control and firpronil insecticide (0.25%) was used as a positive control. The plates with the treatments were left to dry for 4 h, then 10 adults of *R. nigerrimus* were placed in each Petri dish. Each treatment was repeated with 10 Petri dishes. Treated insects were observed daily, and the number of insects killed on each day was recorded to calculate mortality (According to the % mortality Equation 1 in the data analysis section) for five days.

2.4. Polyvinylpolypyrrolidone (PVPP) test to rule out polyphenols

Initially, the extracts of the two species of edible mushrooms were prepared in tubes at 20% concentration and the PVPP compound (0.05 g PVPP/mL) was added to the tubes. These concentrations of extract + PVPP were incubated for 3 h (pvpp has the ability to trap or capture polyphenols). After incubating the fungal extracts, the tubes with the samples were centrifuged and the supernatant was transferred to another tube called polyphenol-free extract.

The pellet containing the PVPP and the polyphenol mixture was discarded. These tubes with the extracts at concentrations of 20% (treated without polyphenols) were evaluated with the respective in vitro tests and their respective controls (Vargas-Magaña, 2013).

2.5. Artemia Salina test to determine toxicity

The *Artemia Salina* assay described by McLaughlin (McLaughlin et al., 1998) was used to evaluate the toxicity of fungal extracts on non-target organisms. The relationship between the concentration of the extracts and the effect it causes on a living organism was quantified (Silbergeld, 2023). Initially, we sought to hatch the *A. salina* cysts; for this, a 500 ml flask containing saline water (35 g/L without iodine), the cysts and a constant air flow of 1 to 2.5 L/m was used. to ensure hatching. The different concentrations of each extract of *P. ostreatus* and *L. edodes* to be evaluated (10, 100, 300, 600 and 1000 µg/mL) were placed in a 24-well Polystyrene culture plate (CORNING®). Subsequently, 1250µL of saline water was placed with 20 nauplii, to have a final volume of 2.5mL per well. In addition, a control group (lugol) was added and these treatments were left to incubate in artificial light for 24 hours. Subsequently, survivors were counted and median lethal concentration (LC50) and toxicity levels were calculated.

2.6. Preliminary mycochemical tests of edible mushroom extracts

Qualitative phytochemical tests individually processed extracts of *P. ostreatus* 1, *P. ostreatus* 2, *L. edodes* 1 and *L. edodes* 2. For these tests, different methodologies were used to qualitatively determine possible functional groups of compounds in the samples of the mushroom extracts (Sarker and Nahar, 2012; Mancilla-Montelongo et al., 2019). In 15mL glass test tubes, 5 mg of extract of each fungus plus the reagents of each qualitative biochemical test were added to observe the colouration change after 5 min. In the Liebermann-Burchard reaction, a 5 mg sample of each fungal extract was placed in glass test tubes, to which 1 mL of acetic anhydride, 100 µL of H_2SO_4 and 10 drops (approximately 10µL) of HCL, respectively, were added. The test was considered positive when the solution acquired a purple colouration, suggesting the presence of triterpenes. In the Shinoda reaction, 1 mL of CH_3OH, 2-3 Mg shavings and 2-3 drops (10µL) of HCL were added to 5mg of each extract, respectively. The test was considered positive for the presence of flavonoids when it changed to red colour. For the Molisch test (carbohy drate), 5 mg of each extract was used, adding 1 mL of water, 2 drops of alpha-naphthol (1%) and 7 drops of H_2SO_4 were added. The test was considered positive if the solution with each extract changed colour to violet, which indicated the presence of carbohydrates. In the reaction to identify coumarins, 5 mg of each mushroom extract was used, to which 1 mL of CH_3OH, 1 mL of alcoholic NaOH and 8 drops of HCL, respectively, were added. The test is considered positive when the colouration of the solutions with each fungal extract plus reagents disappears. For the Wagner reaction, 5 mg of each fungal extract was used, to which an iodine chip, KI and 1 mL of distilled water were added; the change of the solution to a brown colouration indicated the presence of alkaloids. Finally, for the case of the saponins test, it was considered positive when the foam was formed by adding 5mg of each mushroom extract and 1 mL of water (Mancilla-Montelongo et al., 2019).

2.7. Gas chromatography coupled to mass spectrometry

The lyophiliszed extracts of the fungi were suspended in methanol and subsequently injected into the Gas chromatographic (GC). Gas chromatographic analyses coupled to mass spectrometry of the extracts of each fungal sample were carried out on a Thermo Scientific TRACE gas chromatograph (GC) with an ITQ900 ion trap mass detector (Thermo Electron Corporation, Milan, Italy). The carrier gas was helium, with a 1 mL/min flow rate. The column used was TRACE-5MS (30 m, 0.25 µm film and 0.25 mm internal diameter). The program used to analyze of the samples of each fungal extract was a full sweep, started at a temperature of 50 °C, which was maintained for 1 min; subsequently, a temperature ramp was applied with an increase of 7 °C/min up to 300 °C. The temperature of 300 °C was maintained for five minutes. The interface temperature was 280 °C, and the bulk temperature was 200 °C. Signals of the compounds present were detected and compared with the database by NIST17.L library (Páez-Leon et al., 2022).

2.8. Data analysis

The results obtained from the mortality tests of the different treatments at different hours of exposure were analyszed by means of a generaliszed linear model (GLM) to evaluate the differences for the control. *Post hoc*, a Tukey's mean comparison test was applied. The analyses were performed with Statgraphics Centurion XV.

The formula used to obtain the insecticide effect was following (Castañeda-Ramirez et al., 2022b):

$$\%Mortality = \frac{\left(\text{Number of dead insects}\right) x \left(100\right)}{\left(\text{Number of dead insects} + \text{Total number of live insects}\right)} \quad (1)$$

3. Results

3.1. Mortality of R. nigerrimus adults to extracts of P. ostreatus and L. edodes

The highest percentage of mortality utilizing the immersion test of *R. nigerrimus* was 52%, with *P. ostreatus* extract 2 at 20%. The other extracts evaluated did not cause more than 35% mortality. The mortality of *R. nigerrimus* with the polyphenol-free extract of *L. edodes* 2 mushroom increased from 35% to 57%; however, with the other extracts, no differences were observed with the PVPP treatment (Table 1).

The extracts of *P. ostreatus* 1 and *P. ostreatus* 2, showed more significant insecticidal toxicity test on adults of *R. nigerrimus* from a concentration of 20% exposure during 5 days, causing more than 98% mortality in adults of *R. nigerrimus.* The *P. ostreatus* extract 1 at 2.5% caused 73% mortality of *R. nigerrimus* adults. The extract of *L. edodes* 1 showed a mortality of more than 80% of adults of *R. nigerrimus* from a concentration of 5%, while the extract of *L. edodes* 2 at all the concentrations evaluated did not show mortality of even 90%.

In evaluating the extracts without polyphenols (PVPP), only the extract of *L. edodes* 2 showed an insecticidal effect, which decreased the percentage of mortality from 86 to 76% (Table 2).

On the other hand, the values of the concentrations that affect 50 or 90% of the treated insects are observed in summary form, being for the case of the EC_{50} of *P. ostreatus* 1 (1.74%), *P. ostreatus* 2 (2.4%) and *L. edodes* 1 (2.9%) similar according to the confidence intervals (Table 3).

3.2. Artemia Salina testing

Only *P. ostreatus* extract 1 caused 80% mortality of *A. salina* at low concentrations (100 µg/mL). The mortality of *A. salina* was higher than 95% from the concentration of 300 µg/mL of *P. ostreatus* and *L. edodes* extracts (Table 4).

The effective concentrations 50 and 90 of the mortality caused by the extracts against *A. salina* are observed. The extract with the highest activity is *P. ostreatus* 1 with an EC_{50} of 22.07 µg/mL, while the other extracts show similar values. For the case of EC_{90}, the *P. ostreatus* 1 extract stands out more as the one that shows the highest insecticidal activity EC_{90} 121.68 µg/mL for the other extracts (Table 5).

3.3. Mycochemical evaluation

The presence of flavonoids and carbohydrates was not observed in any of the four extracts evaluated (*P. ostreatus* 1, *P. ostreatus* 2, *L. edodes* 1, *L. edodes* 2). In the case of the saponins and coumarins test, a slight presence of saponins was observed in three extracts evaluated (*P. ostreatus* 2, *L. edodes* 1, *L. edodes* 2). The presence of alkaloids was only observed in the extracts of *L. edodes* 1, while the presence of triterpenes was only observed in the extract of *L. edodes* 2.

Table 1. Mortality percentages of *R. nigerrimus* adults during 15 days of exposure to *P. ostreatus* and *L. edodes* extracts by immersion test.

Concentration	Treatments			
	***P. ostreatus* 1**	***P. ostreatus* 2**	***L. edodes* 1**	***L. edodes* 2**
0	20.0±5.83a	21.0±6.7a	22.0±5.3a	15.5±4.0a
2.5%	22.0±5.83a	24.0±6.7ab	17.3±5.3a	22.0±4.0ab
5%	20.5±5.83a	47.5±6.7abc	16.0±5.3a	21.4±4.0ab
7.5%	20.3±5.83a	61.0±6.7c	29.5±5.3a	35.5±4.0b
10%	30.52±5.83a	60.0±6.7c	26.9±5.3a	-
20%	29.0±5.83a	52.0±6.7bc	29.0±5.3a	-
20%-PVPP	32.5±5.83a	51±6.7bc	29.5±5.3a	57.5±4.0c
Fipronil	100.0±5.83b	100±6.7d	100.0±5.3b	100.0±4.0d

[a,b,c,d]Letters show significant differences between columns (P<0.05) (Tukey); (-) Not evaluated due to the low yield of the extract.

Table 2. Mortality percentages of *R. nigerrimus* adults during 5 days of exposure to *P. ostreatus* and *L. edodes* extracts by toxicity test.

Concentration	Treatments			
	***P. ostreatus* 1**	***P. ostreatus* 2**	***L. edodes* 1**	***L. edodes* 2**
0	19.0±3.5a	16.0± 5.06a	27.0±7.4a	24.0±5.1 a
2.5%	73.0±3.5b	48.0± 5.06a	48.0±7.4b	42.0±5.1ab
5%	91.0±3.5c	83.0± 5.06b	85.0±7.4cd	58.0±5.1bc
7.5%	88.0±3.5bc	86.0± 5.06b	72.0±7.4c	86.0±5.1de
10%	95.0±3.5c	93.0± 5.06b	89.0±7.4cd	-
20%	98.0±3.5c	100.0± 5.06b	83.0±7.4cd	-
PVPP	97.0±3.5c	99.0± 5.06b	91.0±7.4cd	76.0±5.1cd
Fipronil	100.0±3.5c	100.0± 5.06b	100.0±7.4d	100.0±5.1e

[a,b,c,d,e] Letters show significant differences between columns (P<0.05); (-) Not evaluated due to the low yield of the extract.

Table 3. Concentration (EC_{50} and $EC_{90\%}$) of the exposure toxicity test against *R. nigerrimus* at 5 days.

Treatments			
***P. ostreatus* 1**	***P. ostreatus* 2**	***L. edodes* 1**	***L. edodes* 2**
CE_{50}=1.748a	CE_{50}=2.469a	CE_{50}=2.922a	CE_{50}=6.089b
(0.76 - 2.75)	(1.56 - 3.15)	(1.67 - 4.21)	(4.94 - 6.84)
CE_{90}=7.626a	CE_{90}=8.249a	CE_{90}=14.028a	CE_{90}=9.202a
(5.42 - 11.05)	(6.53 - 12.52)	(10.21-21.28)	(7.95-13.86)

[a,b] Letters show significant differences between rows (P<0.05).

3.4. Gas chromatography coupled to mass spectrometry

In the case of *P. ostreatus* extract, the most abundant compounds were Methyl tris (trimethyl siloxy) silane (51.95%); Cyclotrisiloxane, hexamethyl- (26.3%); Tris (tert-butyl dimethylsilyloxy) arsane (16.01%). On the other hand, for the compounds detected in the extract of *L. edodes*, Cyclotrisiloxane, hexamethyl- (56.57%) and Tris(tert-butyldimethylsilyloxy) arsane (32.09%) were detected as main compounds. When observing the identified compounds there are 5 compounds present in the two extracts which are: (1) Cyclotrisiloxane, hexamethyl-; 2) Tris(tert-butyldimethylsilyloxy) arsane); 3) Titanium, [(1,2,3,3-.eta.)-2-butenyl](.eta.8-1,3,5,7- cyclooctatetraene)-; 4) 1-Nitro-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid diethylamide; 5) 1,1,1,1,3,5,5,5,5-Heptamethyltrisiloxane (Table 6).

Table 4. Mortality percentages in *Artemia salina* caused by *P. ostreatus* and *L. edodes* extracts.

	Concentration [μg/mL]						
Treatments	**0**	**10**	**100**	**300**	**600**	**1000**	**Lugol**
P. ostreatus 1	12.5±4.0a	30.5±4.0ab	80.0±4.0c	100±4.0c	100±4.0c	100±4.0c	100
P. ostreatus 2	12.5±4.0a	30.5±4.0ab	40.2±4.0b	96.38±4.0c	97.2±4.0c	100±4.0c	100
L. edodes 1	12.5±4.0a	10.0±4.9a	32.08±4.9ab	100±4.9c	100±4.9 c	100±4.9c	100
L. edodes 2	12.5±4.0a	12.9±4.9 a	47.5±4.9 b	97.5±4.9c	100±4.9 c	100±4.9c	100

[a,b, c] Letters show significant differences between rows (P<0.05).

Table 5. Effective concentrations (EC) 50 and 90 of mortality caused by *P. ostreatus* and *L. edodes* extracts against *A. salina*.

Treatments	CE_{50} (μg/mL)	CE_{90} (μg/mL)
P. ostreatus 1	22.07 (15.7- 29.5)a	121.68 (88.3- 182.9)a
P. ostreatus 2	42.25 (25.5- 63.1)ab	364.93 (232.4-685.5)b
L. edodes 1	76.25 (57 - 98.5)b	302.32 (226.4- 438.0)b
L. edodes 2	58.51 (46.1- 72.4)b	268.58 (210.6- 359.8)b

[a,b] Letters show significant differences between columns (P<0.05).

Table 6. Compounds identified in extracts of *P. ostreatus* 1 and *L. edodes* 1 by gas chromatography coupled to mass spectrometry.

No.	RT	Compound	% area	
			P. ostreatus	***L. edodes***
1	10.086	Titanium, [(1,2,3-.eta.)-2-butenyl](.eta.8-1,3,5,7-cyclooctatetraene)-	-	0.43
2	10.663	Titanium, [(1,2,3-.eta.)-2-butenyl](.eta.8-1,3,5,7-cyclooctatetraene)-	0.84	-
3	12.876	Nicotinaldehyde azine	-	0.65
4	20.147	Propanedinitrile, methylene-	0.94	-
5	21.301	Cycloheptasiloxane, tetradecamethyl-	-	5.31
6	22.808	4-Chloro-2-nitrobenzyl alcohol	0.41	-
7	40.73	1-Nitro-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid diethylamide	0.8	-
8	41.586	1-Nitro-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid diethylamide	-	4.48
9	41.769	1,1,1,3,5,5,5-Heptamethyltrisiloxane	-	0.46
10	42.726	1,1,1,3,5,5,5-Heptamethyltrisiloxane	0.58	-
11	48.802	1,2-Benzisothiazol-3-amine, TBDMS derivative	2.13	-
12	49.481	Tris(tert-butyldimethylsilyloxy)arsane	16.01	-
13	50.173	Methyltris(trimethylsiloxy)silane	51.99	-
14	52.196	Cyclotrisiloxane, hexamethyl-	-	56.57
15	53.079	Cyclotrisiloxane, hexamethyl-	26.3	-
16	54.871	Tris(tert-butyldimethylsilyloxy)arsane	-	32.09

(-) absence of the compound; (No.) Number of the order of compounds; (RT) retention time.

4. Discussion

This study evaluated extracts of edible mushroom, *P. ostreatus* and *L. edodes*, against *R. nigerrimus*. The mortality percentages for the immersion test were low (35%). The highest percentage reached was 52% with the *P. ostreatus* 2 extract at 20% concentration, which indicates that these extracts, by this mechanism of action, do not show effects more significant than 50%. This is possibly due to the exposure time with the extract, which is only 20 seconds. On the other hand, in the PVPP test, it was possible to observe which extracts showed insecticidal activity, possibly fusing the polyphenols through the immersion test. When the polyphenols were removed from the extracts, only a change was evidenced in the extract of *L. edodes* 2. The insecticidal activity increased from 35% to 57%, indicating that some polyphenols were blocking the activity of the extract. However, in the case of the other extracts evaluated, their activity was not affected by the polyphenols.

On the other hand, for the second test evaluated "toxicity by exposure", it was possible to observe for the extracts a higher percentage (≥70%) of insecticidal activity in the adults of *R. nigerrimus* at low concentrations (2.5%). This test, unlike the previous one, shows that prolonged exposure at one concentration affected the adults of *R. nigerrimus*. Possibly, this is because the exposure time is longer, and some molecules could enter the adults affecting the insects up to mortality of the insect. In addition, since the four extracts evaluated showed activity in the adults, this test could be considered sensitive for detecting biological material with insecticidal activity. In the case of the extracts without polyphenols, the extract that again showed a difference in activity was *L. edodes* 2, in this case decreasing its insecticidal activity from 86 to 76% mortality, which could indicate that polyphenols have an effect on this extract and its insecticidal activity.

There are few studies on the evaluation of edible mushroom extracts against insect pests; there are no studies specifically against *R. nigerrimus*, nor are there any studies on the insecticidal activity of the fungus *L. edodes*. Therefore, this is one of the first works reporting insecticidal activity of *L. edodes* against *R. nigerrimus*. On the other hand, reported studies of *Pleurotus* against insects have shown insecticidal activity. Recently, a study evaluated *in vitro* extracts of the fungus *P. ostreatus* for the control of the corn weevil (*Sitophilus zeamais* Motschulsky) where the tests used were toxicity by contact and fumigation, repellency and antixenosis. Extracts in ethyl acetate and distilled water showed repellent activity and recorded an antixenosis effect (Pino et al., 2019). This was the first study of *Pleurotus* spp. Fungi against a member of the Curculionidae family, to which *R. nigerrimus* belongs, and the results highlight the importance of evaluating the whole extract. These results support the information found on the activity of *Pleurotus* that could reach more than 90% of insecticidal activity in some tests against some species of the Curculionidae family. In addition, a 2011 study investigated the residual effect of *P. ostreatus* basidiomonads extracts against *Tribolium castaneum* adults. Different extracts and fractions of extracts (petroleum ether fraction, methanol-chloroform extract and hot water) were evaluated. The range of results obtained from the fractions (LD_{50}) was from 1.54 mg/cm^2 to 0.20 mg/cm^2 after 30 hours of exposure (Rahman et al., 2011). These results are similar to the insecticidal activity of the contact toxicity test. Further studies and tests are still needed to determine the activity of fungal extracts, as well as to understand the mechanisms of action and to understand better the volatiles that interacts with *R. nigerrimus* (Espadas-Pinacho et al., 2021).

The evaluation of *A. salina* allows us to know the toxicity of the extract being evaluated and the possible effect that the extract has on other populations, such as those in the marine environment or others (Silbergeld, 2023). The extracts evaluated in this present investigation have 100% mortality activity against *A. salina* at lower concentrations (1000 µg/mL) than those evaluated with the insect *R. nigerrimus*. These differences in concentrations have been shown in studies of insect evaluations against fungi with basidiomata (Castañeda-Ramírez et al., 2022a), where other non-adult stages of insects have been evaluated, and insecticidal effects have been observed at lower concentrations because they are more vulnerable. However, higher concentrations are needed to obtain an insecticidad effect in the adult stage of insects. The *A. salina* test indicates that the extracts evaluated could show insecticidal activity with other genera of insect pests. However, further studies are needed to confirm the concentrations and the activity that the extracts evaluated in the present work could show.

The compounds reported in Table 6 for the *P. ostreatus* and *L. edodes* mushroom extracts have not been reported in other mushrooms of the same genus. However, it is essential to mention that the present work was not a directed study, and the compounds reported are only known to be in the whole extract due to the technique used they are volatiles, but it is not sure that they are the ones that present the activity observed here. There are no reports on the insecticidal activity of the compounds identified in the extracts of *P. ostreatus* and *L. edodes*. However, the Methyltris(trimethylsiloxy)silane has shown antibacterial activity (Balachandar et al., 2022). As for the compound identified as Cyclotrisiloxane, hexamethyl- in the extracts of the two edible mushroom species, it has been reported to have antimicrobial activity against *Staphylococcus aureus* (Priyanka et al. 2015; Ismail et al., 2020). For the case of the other detected compounds, there are still no previous reports of biological activity. More studies are needed to know if these compounds or their mixtures can have insecticidal activity.

On the other hand, the preliminary mycochemical test used helped us to understand the possible groups of compounds present in the evaluated extracts that could not be identified due to the limitations of the mass gas technique used, where complex molecules cannot be detected. The extract of *P. ostreatus* did not show the presence of any specific group; however, here, it could be that other types of molecules were present in this extract with insecticidal activity. On the other hand, different compounds were detected in the extract of *L. edodes*, mainly saponins and coumarins. The chemical profiles of these fungi are complex, so it is necessary to perform directed studies to relate the molecules with the reported activity.

5. Conclusion

The extracts of the fungi *Pleurotus ostreatus* and *Lentinula edodes* showed an insecticidal effect of more than 80% mortality at a concentration of 20% in the exposure toxicity test against *R. nigerrimus* in 5 days. Furthermore, the extracts show their effect in toxicity test against *A. salina* with 100% mortality at 1000 µg/mL concentration.

In addition, the compounds reported by mass gases have shown different biological activity, which could indicate that some of these secondary compounds or their mixtures are responsible for the insecticidal activity. However, there is still a lack of studies to verify which compounds and mixtures may be responsible for the reported insecticidal activity.

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