In vitro EVALUATION OF HYDROALCOHOLIC EXTRACTS OF MYCELIUM, BASIDIOMATA AND SPENT SUBSTRATE OF *Pleurotus ostreatus* AGAINST *Haemonchus contortus* †

[EVALUACIÓN *in vitro* DE EXTRACTOS HIDROALCOHÓLICOS DE MICELIO, BASIDIOMATA Y SUSTRATO DEGRADADO DE *Pleurotus ostreatus* CONTRA *Haemonchus contortus*]

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SUMMARY

Background. The edible mushroom *Pleurotus ostreatus* is a potential alternative for the control of gastrointestinal nematodes due to their nematoxins and secondary metabolites. **Objective.** To evaluate the *in vitro* anthelmintic activity of hydroalcoholic extracts of mycelium, basidiomata and spent substrate of the edible mushroom *P. ostreatus* against eggs and unsheathed larvae of *Haemonchus contortus*. **Methodology.** The mycelium of *P. ostreatus* was extracted with ethanol–water (70:30) and the basidiomata and the spent substrate with methanol–water (70:30) which were concentrated to obtain the mycelium (MHA), basidiomata (BHA) and spent mushroom substrate extract (SMSE) extracts. The eggs and larvae of *H. contortus* were exposed to different extracts in 96-well plates. The tests used were the egg-hatch and larval mortality tests. Three exposure times, 24, 48 and 72 h, were considered. The data were analyzed with a factorial design and the comparison of means by Duncan test. **Results.** The percentage anthelmintic effectiveness of the mycelium hydroalcoholic extract (MHA) of *P. ostreatus* against eggs and larvae of *H. contortus* were 93 and 96.8% at 12.8 and 240 mg/mL (respectively) after 72 h. Also, activity was observed for SMSE and BHA against eggs, with values of 87.2 and 100%, respectively, at 25.6 mg/mL after 72 h. However, SMSE showed no activity against *H. contortus* larvae, while the BHA extract showed less than 70% corresponding activity. **Implications.** This was an exploratory study of *in vitro* tests; the *in vivo* studies are still needed. **Conclusion.** The MHA extract of *P. ostreatus* showed anthelmintic activity against the eggs and larvae of *H. contortus.*

Keywords: Anthelmintic; extracts; Larval Mortality Test; Egg Hatch Test; *Haemonchus contortus; Pleurotus ostreatus.*

RESUMEN

Antecedentes. El hongo comestible *Pleurotus ostreatus* es una alternativa potencial para el control de los nematodos gastrointestinales debido a sus nematoxinas y metabolitos secundarios. **Objetivo**. Evaluar la

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actividad antihelmíntica *in vitro* de los extractos hidroalcohólicos de micelio, basidiomata y sustrato degradado de los hongos comestibles *P. ostreatus*, contra huevos y larvas sin vaina de *Haemonchus contortus*. **Metodología.** El micelio de *P. ostreatus* se extrajo con etanol: agua (70:30) y por otro lado los basidiomas y el sustrato degradado con metanol: agua (70:30, los cuales se concentraron para obtener los extractos MHA, BHA y SMSE respectivamente. Los huevos y larvas de *H. contortus* fueron expuestos a diferentes extractos en placas de 96 pozos. Se consideraron tres tiempos de lectura 24, 48 y 72 h. Los datos fueron analizados con un diseño factorial y la comparación de medias con la prueba Duncan. **Resultados.** Los resultados del porcentaje de eficacia antihelmíntica del extracto hidroalcohólico de micelio (MHA) de *P. ostreatus* contra los huevos y larvas de *H. contortus* fueron de 93 y 96.8% a 12.8 mg/mL y 240 mg/mL a 72 h (respectivamente). También se observó una actividad del extracto de sustrato degradado (SMSE) y del extracto de basidiomata (BHA) contra los huevos con un valor de 87.2 y 100 % a 25.6 mg/mL a las 72 horas. Sin embargo, el SMSE no mostró actividad contra larvas de *H. contortus.* Mientras que el extracto BHA mostró menos del 70% de actividad para las larvas de *H. contortus*. **Implicaciones**. Las pruebas evaluadas siguen siendo estudios *in vitro*, falta realizar estudios *in vivo.* **Conclusión.** El extracto de MHA de *P. ostreatus* mostró actividad antihelmíntica contra los huevos y larvas de *H. contortus.*

Palabras clave: Antihelmíntico, Extractos, *Haemonchus contortus*, *Pleurotus ostreatus.*

INTRODUCTION

Parasitic diseases caused by gastrointestinal nematodes (GIN) are a major problem in ovine farming. One of the most important species is *Haemonchus contortus* due to its high prevalence (Hoste *et al.,* 2016). Currently, chemical treatments are used to control parasites. However, the indiscriminate use of these drugs has generated anthelmintic resistance and also contamination of the environment by residues in meat and milk, thus affecting human health, damaging other organisms and resistance problems (Aparicio-Medina *et al.*, 2011, Torres-Acosta *et al*., 2012, Aguilar-Marcelino *et al.*, 2014, Encalada-Mena *et al.*, 2014). Alternative methods of GIN control are currently being sought. Several studies have been conducted with natural products containing secondary compounds with activity against gastrointestinal nematodes (Sandoval-Castro *et al.*, 2012; Macedo *et al.,* 2010; Zhu *et al.*, 2013). Fungi of the genus *Pleurotus* spp. have shown nematocidal activity, which has been attributed to a nematotoxin (such as *trans*-2-decenedioic acid; Kwok *et al.*, 1992). The edible mushroom *Pleurotus ostreatus* is a potential alternative for the GIN control by nematotoxins and secondary metabolites that are produced (Kwok *et al.*, 1992). At present, the use of secondary metabolites from organic extracts from the mycelium and basidiomata of *P. ostreatus* against phytopathogenic nematodes and animal parasites has been reported (Palizi *et al.*, 2009, Comans-Pérez, 2014, Arizmendi 2014**)**. Edible fungi have metabolites with different medicinal and therapeutic properties, such as anti-cancer, antimutagenic, antidiabetic, immunoprotective, antiviral, antimicrobial, antioxidant, anticholesterolemic, immunomodulatory, antihypertensive, insecticidal and antiparasitic (nematicidal) activities (Wasser, 2011). This type of extract (70–30% ethanol or methanol–water solution) was used because anthelmintic activity of macromycetes fungi has previously been reported with these solvents (Pineda-Alegría *et al.*, 2017). Therefore, the objective of this research was to evaluate the *in vitro* hydroalcoholic extracts of mycelium, basidiomata and degraded substrate of the edible mushroom *P. ostreatus* against eggs and larvae (L3) of the parasitic nematode of sheep, *H. contortus.*

MATERIALS AND METHODS

Location

This research was carried out at the facilities of the National Center for Disciplinary Research in Animal Health (CENID-SAI) of the National Institute of Forest, Agricultural and Livestock Research (INIFAP), at the Polytechnic University of the State of Morelos (UPEMOR) and at the Center for Biomedical Research of the South (CIBIS-IMSS). All these institutions are located in the State of Morelos, Mexico.

Obtention of mycelium, basidiomata and degraded substrate of *P. ostreatus*

Mycelium production. The strain (HEMIM-50) is wild in origin from the north of the State of Morelos, Mexico and belongs to the mycology strain of the Biology Research Center of the Autonomous University of the State of Morelos. The strain was extracted directly from the inoculum in an agar medium of whole wheat flour (WWF) for eight days. Subsequently, two cuts of 4 mm^2 each were made and transferred into other Petri dishes (n = 10) with WWF medium (100 × 15 mm),

leaving a growth period of 10 days for the harvest of mycelia (Comans-Pérez, 2014).

Production of basidiomata and spent mushroom substrate

The spent mushroom substrate is made up of wheat straw previously treated with boiling water and placed in plastic bags (2 kg). Subsequently, inoculation was performed with 200 g of *P. ostreatus* mycelium, with a uniform distribution throughout the substrate and incubated at a temperature of 25–30 °C, relative humidity 70–80% with poor lighting, for the development of the primordium. The harvest period for basidiomata was 30–40 days (Quimio, 2002). The spent mushroom substrate or waste material, i.e., the wheat straw where the fungal material grew, was collected for later extraction with the basidiomata and mycelium (Comans-Pérez, 2014).

Preparation of hydroalcoholic extracts

The hydroalcoholic extract of *P. ostreatus* mycelium consisted of a 70:30% ethanol–water solution. For the hydroalcoholic extracts of basidiomata and spent mushrooms substrate *of P. ostreatus*, a 70:30% methanol–water solution was used. Each material (mycelium, basidiomata and degraded substrate) was placed in 125 mL Erlenmeyer flasks and the hydroalcoholic solution added until the entire fungal material was covered, then left to rest for 24 h. The material was filtered (Whatman No. 5) and distilled in a rotary evaporator (Heidolph G3, Germany), to obtain the extract of mycelium (MHA), basidiomata (BHA) and the spent mushroom substrate (SMSE). All were lyophilized (Pineda-Alegría *et al.*, 2017).

Production of larvae of *Haemonchus contortus*

The *H. contortus* strain "Hueytamalco" belonging to the CENID-SAI Helminthology Department, INIFAP Mexico, was used. This strain was isolated from the feces of a sheep naturally infected with *H. contortus* in the municipality of Hueytamalco, Puebla State, Mexico. The strain has been identified morphologically and molecularly (Liébano, 2004).

The *H. contortus* eggs were obtained from a donor sheep of 25 ± 1 kg, which were kept under controlled housing and feeding conditions. This lamb was experimentally infected with *H. contortus* infecting larvae (L3) at a single dose of 350 larvae per kg live weight, orally. After a period of 21 days, feces were collected directly from the rectum of the sheep. McMaster technique was used to determine fecal counts per gram of stool (Bauer *et al.* 2010). In order to obtain larvae, coprocultures were made and placed in plastic containers and mixed with polystyrene particles. Stool cultures were incubated for 6 days at 28 °C and then larvae were recovered using the Baermann funnel technique (Castañeda-Ramírez *et al.*, 2017).

Egg-hatch test

The feces of the donor animal were collected and macerated until a homogeneous mixture was obtained and this mixture then sifted (# 40, 100, 200 and 400). The sieved sample was recovered in a 250 mL beaker. Afterwards, washing was carried out, part of the sample was placed in 15-mL tubes and sucrose added at 20% (1:4), a band was formed by density where the eggs were found, which were collected. This sample of eggs was washed with water and finally quantified to determine the egg concentration (Pineda-Alegría *et al*., 2017).

Plates of 96 wells were used to perform the bioassays; into these wells were initially placed 50 µL of the concentrations (25.6, 12.8, 6.4, 3.2, 1.6 and 0.8 mg/mL) of each extract with their respective positive and negative controls (distilled water and benzimidazole 10 mg/mL), each with four replicates. Subsequently, 50 µL of the egg suspension (200 eggs per well) were placed into each well. This plate was left in incubation at minimum 28 °C for 48 h to achieve hatching. The hatched larvae and unhatched eggs in each well observed and counted by optical microscope (10X and 40X). This bioassay was carried out at 48 and 72 h post treatment. After some time, 10 µL lugol was added to each well to stop the hatching.

Elimination of the sheath of *H. contortus* infecting larvae with sodium hypochlorite solution

The bioassay was by exposure of larvae to the extracts and observing the induced mortality. This bioassay is based on the larval motility test (Castañeda-Ramírez *et al.*, 2017) with some modifications to the original test concerning exposure time and using unsheathed larvae, since the susceptibility of larvae without sheaths is considered higher (Conder and Johnson, 1996). For the unsheathing of the *H. contortus* L_3 larvae, sodium hypochlorite 0.187% was used for 5 min, verifying the unsheathing in an aliquot; three washes were then carried out with distilled water and the contents centrifuged at 3500 rpm for 3 min. After the final wash the supernatant was discarded,

and the larvae recovered without liquid and re-suspended.

Larval mortality bioassay against unsheathed larvae

In the plates of 96 wells three treatments of four repetitions each were formed, containing 80 µL of the different concentrations of each extract (240, 200, 160 and 80 mg/mL); negative and positive controls were also placed in each plate (distilled water and ivermectin 10 mg/mL). Subsequently, 20 µL of distilled water containing 200 L_3 larvae of *H. contortus* without sheaths were added. This confrontation of extracts and larvae was incubated for 24, 48 and 72 hours to be read under a compound microscope (4X and 10X), where alive and dead larvae were observed and quantified.

Statistical analysis

The mortality (proportion) was transformed to square root and then to arcsine in order to homogenize the variance and obtain an approximation to the normal distribution (Richardson and Overbaugh, 2005; Vidyashankar *et al.*, 2007). Data on the egg-hatch test were analyzed through a completely random factorial design, where factor A was the product dose (25.6, 12.8, 6.4, 3.2, 1.6, 0.8 mg/mL and control), factor B was the reading time (48 and 72 h) and factor C was the type of hydroalcoholic extract (mycelium, basidiomata and spent substrate) using SAS proc GLM.

For larval mortality, a variance analysis was performed to determine the mortality rate using the three products (mycelium, basidiomata and spent substrate), three reading times (24, 48 and 72 h), and four doses (240, 200, 160 and 80 mg/mL, positive and negative controls). The comparison of means was made with Duncan's test ($\alpha = 0.05$).

Finally, the effective concentration (EC 50%) was calculated using the Probit program (Polo plus).

RESULTS

Egg-hatch test

The inhibition of the three *P. ostreatus* extracts against *H. contortus* eggs evaluated are presented in **Table 1**. For the MHA extract of *P. ostreatus* against eggs of *H. contortus*, an inhibition of hatching of more than 98% from the lowest concentration to the highest concentration evaluated (0.8 to 12.8 mg/mL) was observed at the two incubation times of 48 and 72 h. Also, for the BHA extract from the lowest concentration, hatching inhibition was observed of 98.2 and 66.6% at 48 and 72 h, respectively.

On the other hand, SMSE extract resulted in an anthelmintic activity at 48 and 72 h of 77 and 87.2%, respectively, on the hatching of *H. contortus*.

For EC50 were not calculated for all the extract because some extracts had a very high anthelmintic activity at the lowest concentrations. The EC50 values and their confidence intervals obtained at 72 h were 0.5 mg/mL (0.362–0.712) and 9.6 mg/mL (6.8–12.4) for the BHA and SMSE extracts, respectively. At 48 h the EC50 value for SMSE was 24 mg/mL (15.9–49.3).

Table 1. Percentages of inhibition (mean and SE) of hatching of *Haemonchus contortus* eggs exposed to hydroalcoholic extracts of *Pleurotus ostreatus* evaluated at different incubation times.

Extract	**MHA**		**BHA**		**SMSE**	
mg/mL	48 h	72 h	48 h	72 h	48 h	72 h
Water	10 ± 2.6 ^{B}	8 ± 2.1 ^{B}	4.9 ± 1.4 ^{C}	3.8 ± 10.6 ^{C}	6.5 ±1.7 ^{D}	7 ± 2.1 ^{D}
0.8	100 ± 2.7 ^{A}	100 ^{A}	98.2 ± 1.4 ^{B}	66.6 ± 10.6 ^{B}	28 ± 7.6 ^{C}	18.2 ± 11.5 ^{C}
1.6	98.9 ± 0.87^{A}	100 ^{A}	99.8 ± 1.4 ^{A}	97.9 ± 10.6 ^{A}	20 ± 7.2 ^{C}	18 ± 8.8 ^{C}
3.2	100 ^{A}	100 ^{A}	100 ^{A}	98.1 ± 10.6 ^{A}	29 ± 5.8 ^{C}	41 ± 10.3 ^{B}
6.4	98.7 ± 1.2 ^{A}	91.7 ± 1.5 ^{A}	100 ^{A}	99.2 ± 10.6 ^{A}	46 ± 7.8 ^{B}	46.3 ± 14.8 ^{B}
12.8	98.6 ± 0.8^{A}	93.9 ± 6.9 ^{A}	100 ^{A}	100 ^{A}	42 ± 14.5 ^{B}	39.5 ± 9.7 ^{B}
25.6	-	-	100 ^{A}	100 ^{A}	77 ± 6.8 ^{A}	87.2 ± 5.5 ^{A}
AH	100	100	100	100	100	100

Extract of mycelium (MHA), basidiomata (BHA) and spent mushroom substrate (SMSE).
AH: Anthelmintic: benzimidazole
$^{A, B, C, D}$ Letters indicate differences within columns.

Table 2. Mortality percentages of exsheated *Haemonchus contortus* larvae exposed to hydroalcoholic extracts of *Pleurotus ostreatus.*

	Incubation time		
Concentration (mg/mL)	**24 h**	**48 h**	**72 h**
MHA			
Water	0.5 ± 0.6 ^{D}	0.1 ± 0.5 ^{D}	0 ^{D}
80	6 ± 1.7 ^{C}	14 ± 6.5 ^{C}	35.3 ± 23 ^{C}
160	9.8 ± 2.5 ^{B}	41.3 ± 5.5 ^{B}	72 ± 12.8 ^{B}
200	-	-	-
240	17 ± 5 ^{A}	74.8 ± 2.1^{A}	96.8 ± 2.21^{A}
AH	100	100	100
BHA			
Water	0 ^{D}	8.2 ± 2.4 ^{C}	1.5 ± 0.5 ^{C}
80	13 ± 2.5 ^{B}	6 ± 2.9 ^{B}	23.3 ± 18.8 ^{B}
160	18 ± 5.2 ^{A}	30.3 ± 12 ^{A}	62.3 ±7.2 ^{A}
200	-	-	-
240	11.8 ± 5.4 ^{C}	50.5 ± 21^{A}	70.5 ± 14.9 ^{A}
AH	100	100	100
SMSE			
Water	0 ^{B}	18 ± 2.4 ^{A}	1.5 ± 0.5 ^{B}
80	-	-	-
160	-	-	-
200	7.3 ± 3.5 ^{A}	2.5 ± 3.1 ^{B}	11.3 ± 2.2 ^{A}
240	-	-	-
AH	100	100	100

Extract of mycelium (MHA), basidiomata (BHA) and spent mushroom substrate (SMSE).
AH: anthelmintic
$^{A, B, C, D}$ Letters indicate differences within columns and same extracts
– not evaluated

Test against unsheathed larvae

Table 2. Shows the anthelmintic activity of the extracts against unsheathed larvae. The MHA extract showed no activity at 24 h; however, at 48 and 72 h, it showed the highest activity of the three extracts evaluated: this activity was at the highest concentrations evaluated (240 mg/mL). The extract with the lowest activity against larvae was the SMSE extract with 11.3% evaluated at a concentration of 200 mg/mL at 72 h.

The EC50 values of the extracts (MHA, BHA and SMSE) at 24 h post treatment could not be calculated due to the low anthelmintic activity; the same was the case at 48 and 72 h for the SMSE extract. For 48-h post-treatment the EC50 values and their confidence intervals were 169 mg/mL (159.6–179.8), 242.1 mg/mL (224.7–266.7) for MHA and BHA, respectively. Finally, at 72 h, the values were 103.2 mg/mL (93.3–112.5) and 140.9 (128.4–154) for the MHA and SMSE extracts, respectively.

DISCUSSION

The frequent and indiscriminate use of drugs for the control of GIN has resulted in the problem of anthelmintic resistance, which is a growing global threat (Encalada-Mena *et al.*, 2014; Chan-Pérez *et al.*, 2016). Some products, such as macrocyclic lactones, cause multiple damage to beneficial organisms found in the soil, such as the dung beetle (*Circellium bacchus*) (Basto-Estrella *et al.*, 2012, Basto-Estrella *et al.*, 2016), so it is urgent and necessary to look for different and sustainable control alternatives (Aguilar-Marcelino *et al.*, 2017, Rodríguez-Martínez *et al.*, 2018). In recent years edible fungi have been reported to have multiple biological functions and medicinal properties (Sánchez and Mata, 2012) and be "nutraceutical" foods (Sekan *et al.*, 2019). Therefore, the present study evaluated *in vitro* the hydroalcoholic extracts of mycelium, basidiomata and degraded substrate of the edible fungus *P. ostreatus* against eggs and larvae of the parasitic nematode of ovines *H. contortus* (L_3), seeking to

obtain a new biological alternative for the control of nematodiasis and thus counteract the use of chemicals in agricultural systems.

Egg-hatch test for *H. contortus* eggs

The best egg activity was for the MHA extract, where mortality of more than 98.6 % at 0.8 mg/mL was observed. In previous studies, Cedillo (2016) carried out a targeted study of the mycelium hydroalcoholic extract of the fungus *P. ostreatus* (ECS-1123) against *H. contortus* eggs and reported a 100% inhibition activity at 1.25 mg/mL. The metabolite identified as responsible was xylitol. This is evidence that *P. ostreatus* mycelium has anthelmintic activity against *H. contortus* eggs. In addition, other molecules with anthelmintic activity have already been detected in edible mushrooms, such as a new peptide called "omfalotine" isolated from the fungus *Omphalotus olearius*; this is a peptide that has demonstrated a nematicidal activity similar to ivermectin (Mayer *et al*., 1997; Li and Zhang, 2014).

The results of the BHA evaluation ranged from 66 to 100% in the evaluated concentrations of *P. ostreatus.* There are no studies on the hatching test for this fungus; however, it showed a high ovicidal effect. There are reports of the same genus of fungus, where in a previous study (Pineda-Alegría *et al.*, 2017), the hydroalcoholic extract of the basidiomata of *P. djamor* was evaluated against eggs and a 9.8% inhibition against eggs was found (at 625 µg/mL), but for another fraction a 100% inhibition was reported at 72 h at 40 mg/mL. This may indicate that *P. ostreatus* could have different or a higher number of metabolites responsible for anthelmintic activity against *H. contortus* eggs.

Additionally, the result of the BHA in the inhibition of *H. contortus* eggs at 48 h was probably due to a rapid and powerful activity due to the secondary metabolite products present in BHA.

With respect to the SMSE extract, a nematicidal activity higher than 80% at 25.6 mg/mL and 72 h was observed. Díaz-Rodríguez (2015) reported that the hydroalcoholic extract of substrate of *P. ostreatus* against eggs of *H. contortus* showed an activity of 99.35% inhibition at 0.5 mg/mL at 72 h, different from the results obtained in this study. Reported studies show the importance of *P. ostreatus* extracts and it is not ruled out that there is a potential metabolite responsible for the anthelmintic activity.

The use of different solvents to obtain the extracts may be another factor in the variability of the results obtained. In this case the mixtures used were ethanol–water (70:30) for the MHA extract and a methanol–water mixture (70:30) for the BHA and SMSE extracts. High-polarity mixtures, such as methanol, ethanol or acetone–water, are used to extract a large amount of plant metabolites. However, differences have been observed in the amount of polyphenols and condensed tannins extracted with each of these solvents (Hernández-Bolio *et al.*, 2018).

In this study it was observed that extracts of similar polarity produce significantly different anthelmintic effects, even when obtained from the same plant material. The MHA extract was the best in terms of anthelmintic activity against eggs and larvae, while the SMSE extract was the one with the lowest anthelmintic activity against eggs and larvae. In general, the extracts showed higher activity against *H. contortus* eggs. A similar effect was described by Castañeda-Ramírez *et al.* (2020), who showed that methanolic extracts from anonaceae leaves have a high anthelmintic effect against *H. contortus* eggs (ovicidal and hatch-blocking effects) but a poor inhibitory effect on the shedding of infecting larvae. The acetone–water extracts of those same plant material have a poor ovicidal effect but can prevent the hatching of the larvae formed inside the eggs and have a clear inhibitory effect on the shedding of the infecting larvae of *H. contortus*.

Larval mortality

The MHA extract showed activity of 96.8% at 240 mg/mL and 72% at 160 mg/mL. These results are similar to those indicated in the hydroalcoholic extract of the mycelium of the fungus *P. ostreatus* (0152) with an activity of 86% at 200 mg/mL against *H. contortus* infecting larvae at 72 h (Comans, 2014; Comans-Pérez *et al.*, 2021). The similarity may be due to the fact that both mycelial extracts were from the same species and used the same type of extraction. However, it is important to mention that the origin of the strains was different; the strain used in the present study was isolated from the north of the State of Morelos, and the strain of Comans (2014) was from Chiapas. On the other hand, in a study of the mycelium of the fungus *P. ostreatus*, larvae of *H. contortus* were evaluated and a 26% mortality was observed at 72 h (Cedillo, 2016). In this study it was observed that in the fractions of *P. ostreatus* evaluated against larvae, anthelmintic activity decreased.

The BHA extract of the fungus *P. ostreatus* showed activity greater than 70% at 240 mg/mL after 72 h of confrontation. Previous studies report that the hydroalcoholic extract of the basidiomata of the edible mushrooms *P. ostreatus* (ECS-1123) presented lower than 50% mortality of unsheathed *H. contortus* infecting larvae at 72 h post-confrontation at 80 mg/mL (Arizmendi, 2014). Inhibition values against larvae were different because in the present study, a higher concentration was used to induce larval mortality. On the other hand, another study of the basidiomata of *P. djamor* against larvae of *H. contortus* found a 77.67% mortality against larvae at 160 mg/mL. In addition, evaluating another fraction, a 90% mortality was found at 72 h at 40 mg/mL (Pineda-Alegría *et al*., 2017). This study shows that the raw extract did not present a good activity against larvae, possibly because of the species of fungus evaluated. However, a greater anthelmintic action was observed in the fraction. The results found by Pineda-Alegría *et al*. (2017) against larvae and those of the present study are very similar in terms of the activity found at 160 mg/mL at 72 h, despite using different species of the edible mushrooms *Pleurotus*.

The SMSE extract evaluated against *H. contortus* larvae did not show anthelmintic activity, although hydroalcoholic extracts of the degraded substrate of *P. ostreatus* had already been reported to show activity against *H. contortus* larvae, with 47% inhibition at 20 mg/mL after 72 h (Díaz-Rodríguez, 2015). These differences could be a reflection of the type of extraction, the substrates evaluated and the fungus strain, among other variables.

Not only are there studies of this type of edible mushroom against animal parasites, but it has also been reported that *P. ostreatus* produces a toxin that reduces the size of the anterior part of the free-living nematodes of the Diplogastridae family (Satou *et al.*, 2008). The nematicidal effect of *P. ostreatus* and *P. tuberregium* has been reported by Okorie *et al.* (2011) against the gill nematode *Meloidogyne incognita* (J_2) in three different varieties of bean seedlings (*Glycine max* L. Merril), increasing the growth of the seedlings and decreasing the number of gills in the root system of the seedlings treated with the edible mushrooms compared to the control group. This highlights the biological activity of *P. ostreatus* in this study and future research.

CONCLUSION

Of the three materials evaluated, the MHA extract of *P. ostreatus* showed the highest anthelmintic activity against the larvae and eggs of *H. contortus*.

The reason for different metabolites in the different extracts is the normal allocation of the secondary metabolites along the organism body (plant or fungi), which is well known.

Due to the type of anthelmintic activity shown by the fungal extracts against larvae of *H. contortus*, it is necessary to look for different *in vitro* methodologies to evaluate the effects of the extracts against larvae.

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Compliance with ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant Mexican guidelines regarding animal welfare and unnecessary animal suffering; these are Good Management Practices policies well established at our institution. The Norma Oficial Mexicana (Official Rule Number) NOM-051-ZOO-1995 (http:/www.senasica. gob.mx) as well as the Ley Federal de Sanidad Animal (Federal Law for Animal Health) DOF 07-06-2012 (http:/www.diputados.gob.mx/LeyesBiblio/ref/lfsa.htm) were strictly abided to and all the procedures performed in studies involving animals were in accordance with the ethical standards at INIFAP.

Data availability. Data are available with <Liliana Aguilar-Marcelino, aguilar.liliana@inifap.gob.mx> upon reasonable request.

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