IN VITRO LETHAL CAPABILITY OF TEN STRAINS OF EDIBLE MUSHROOMS AGAINST *HAEMONCHUS CONTORTUS* (NEMATODA) INFECTIVE LARVAE

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ABSTRACT

Haemonchus contortus is considered to be the most pathogenic haematophagous gastro-intestinal parasitic nematode in small ruminants. The most common control method for this and other nematodes is the continuous administration of chemical anthelmintic (AH) drugs in the host animals; however, the indiscriminate use of these products has triggered some problems including anthelmintic resistance. New alternatives of control are necessary to diminish the use of AH drugs. This study was conducted to evaluate the *in vitro* nematicidal activity of mycelia from ten strains of edible mushrooms against *Haemonchus contortus* infective larvae. This study was carried out using water agar plates. Eleven groups of plates were established: One control (without mycelium) and ten groups each containing mycelia of each selected fungi. Five hundred *H. contortus* L_3 were deposited on each plate (n=10) and incubated at 18-25 °C, for 5 days. The highest lethal effects (82-99%) were recorded with *P. ostreatus* ECS-1123 and ECS-0152, *P. eryngii* ECS-1292, *P. cornucopiae* ECS-1328 and ECS-1330 and *L. edodes* ECS-0401.

Keywords: *Haemonchus contortus*, edible mushrooms, biological control

INTRODUCTION

Gastrointestinal parasitic nematodes (GIN) severely affect animal health and diminish the productive potential of cattle and small ruminants leading to severe economic losses worldwide [1]. Global sales of anti-parasitic compounds are estimated at approximately tens of billions of dollars [2].Worldwide, the most common GIN genera spread around the world are *Haemonchus*, *Trichostrongylus*, *Teladorsagia*, *Nematodirus* and *Cooperia* [3]. The main method of control of these parasites is chemotherapy, which consists of the regular administration of chemical anthelmintic drugs to the animals to diminish the parasitic burden and its health consequences; however, the indiscriminate and continuous use of these products has resulted in anthelmintic resistance (AR) by GIN throughout the world [4,5].

Furthermore, other problems derived from the use of chemical anthelmintic drugs have been observed; for example, animal products and sub-products (meat, milk and wool) for human consumption can contain residues of such drugs, thus constituting a public health risk [6]. Additionally, some active anthelmintic drug molecules are eliminated through feces of treated animals to the soil, causing harmful effects on beneficial organisms and putting the environment at risk [7]. Therefore, there is a need for the development of alternative and complementary methods that reduce the use of chemical antiparasitic drugs.

Researchers throughout the world have become increasingly interested in alternative methods of control in order to diminish the use of chemical strategies against parasites. These include the use of plants with anthelmintic activity [8], vaccines [9] and the use of natural nematode antagonists such as nematophagous fungi. Edible mushrooms have been used by ancient cultures as natural medicines such as antioxidants [10], antitumoral [11]. Recent studies have demonstrated that some species of edible mushrooms such as the *Pleurotus* genus possess nematicidal activity through the production of a nematoxin which is able to inhibit the nematode movement allowing hyphal penetration and finally digesting the body by enzymatic action [12]. Such biological activity could be the result of a self-defense mechanism in mushrooms which acts against the attack of myceliophagous nematodes. Research into this mechanism could lead to the obtention of elite molecules which could be developed into natural antiparasitic products [13,14]. This research was aimed to evaluate the *in vitro* nematicidal

activity of mycelia of ten strains of edible mushrooms against the infective larvae of the sheep parasitic nematode *Haemonchus contortus*.

MATERIALS AND METHODS

The biological material used included mycelia of the following fungi: *Pleurotus ostreatus* ECS-0152 and ECS-1123, *P. eryngii* ECS-1290 and ECS-1292, *P. cornucopiae* ECS-1328 and ECS-1330, *Coprinus comatus* ECS-1103, *Panus* sp. ECS-801, *Lentinula boryana* ECS-0402 and *L. edodes* ECS-0401 from the Tropical Mushrooms Laboratory of El Colegio de la Frontera Sur (ECOSUR), in Tapachula, Chiapas, Mexico. The *in vitro* confrontation of the different fungal strains was carried out in Petri dishes (60x15 mm) containg 2% water-agar. Eleven series of 10 water agar plates each were implemented. The age of selected fungal cultures was 20 days. The first series was considered the negative control containing only nematode larvae without any fungi. Five hundred *H. contortus* infective larvae (L_3) were added to each dish in each series, and incubated at 18-25 °C for 5 days.

Recovery of total larvae (live or dead) from the plates was performed by placing the content of each plate on the Baermann funnel for 12 h. Larvae were quantified and means of recovered larvae were compared with the mean of recovered larvae from control. The mean number of total alive larvae recovered from the control series was considered as 100% viability. The lethal activity of fungi was expressed as percentage mortality. Data were square root transformed $\sqrt{x+0.5}$ and a completely randomized design was used. An ANOVA analysis followed by the Tukey test ($\alpha = 0.05$) was used. The mean number of dead larvae recovered in each treatment was considered as the dependent variable. SAS statistic software was used for the analysis. Percentage mortality was estimated using the following formula:

$$\% \text{ Mortality} = \frac{\bar{x} \text{ Control} - \bar{x} \text{ Treated}}{\bar{x} \text{ Control}} \text{x } 100$$

Where: X Control = Mean of *H. contortus* larvae recovered from control group;

X Treated= Mean of *H. contortus* larvae recovered from treated group.

RESULTS

Results showing the mean numbers and standard deviations of *Haemonchus contortus* (L_3) recovered larvae after confrontation with mycelia of the different edible mushrooms, in addition to the variant coefficient (%) and larval mortality percentage are shown in Table 1.

Note: Same small letters in the mortality column indicate that the values were not statistically different, according to Tukey's test ($\alpha = 0.05$), $\bar{x}$ =Average L_3 alive; SD= Standard deviation.

The nematicidal activity of the fungal mycelia against *H. contortus* L_3, ranged from 4.8% to 99.6% mortality. The strains *L. boryanus* ECS-0402 and *C. comatus* ECS-1103 presented 61 and 56.3% mortality; respectively. The highest nematocidal activity corresponded to *L. edodes* ECS-0401 (99.6%), *P. eryngii* ECS-1292 (98.1%), *P. ostreatus* ECS-1123 (97.2%), *P. cornucopiae* ECS-1328 (92.9%), *P. ostreatus* ECS-0152 (92.1%), *P. cornucopiae* ECS-1330 (85.9%) and *P. eryngii* ECS-1290 (82.2%). It is worth mentioning that once the plates were revised at the end of the incubation period some larvae were observed to be motionless with their bodies experiencing no apparent changes and maintaining their normal integrity.

In some cases, after adding some water drops or applying a physical stimulus with a fine needle, some larvae were observed to be actively moving on the agar surface. The bodies of other motionless larvae were observed to have been invaded by some of the assessed mushroom strains; this finding was most evident particularly in three species of the evaluated mushroom mycelia: *Pleurotus cornucopiae* (strain 1328), *P. cornucopiae* (strain 1330) and *Lentinul aboryanus* (strain 402). A set of selected microphotographs showing some larvae invaded by mushroom mycelia of these species are shown in Fig. 1.

Table 1. Mean number and standard deviation of *Haemonchus contortus* (L_3) recovered larvae after 5 days *in vitro* confrontation with mycelia of different edible mushrooms, and larval mortality percentage.

Strain	$\bar{x}$± (SD)	Mortality (%)
Pleurotus ostreatus ECS-1123	14±(18.4)	97.2[c]
Pleurotus ostreatus ECS-0152	38±(39.3)	92.1[c]
Pleurotus eryngii ECS-1292	6±(9.6)	98.1[c]
Pleurotus eryngii ECS-1290	68±(45.4)	82.2[c]
Pleurotus cornucopiae ECS-1328	34±(28.3)	92.9[c]
Pleurotus cornucopiae ECS-1330	78±(41.5)	85.9[c]
Coprinus comatus ECS-1103	144±(63.8)	56.3[b]
Panus sp. ECS-801	314±(58.1)	4.8[a]
Lentinula boryanus ECS-0402	184±(123)	61.8[b]
Lentinula edodes ECS-0401	2 ±(6.2)	99.6[c]

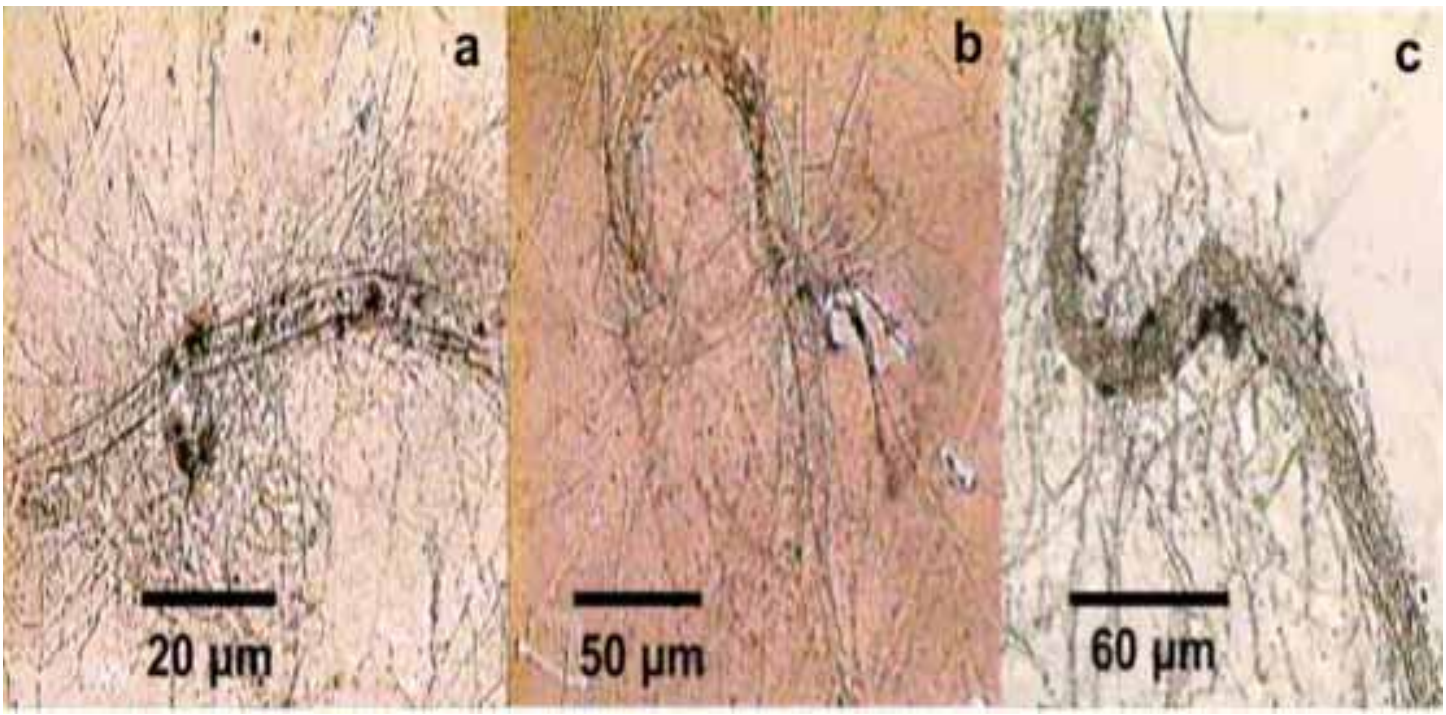

Figure 1. Microphotographs showing the aspect of *Haemonchus contortus* infective larvae (L_3) invaded and degraded by mycelia of 3 edible mushroom, after 5 days *in vitro* confrontation on water agar plates at 25 ºC. a) *Pleurotus cornucopiae* (strain 1328); b) *P. cornucopiae* (strain 1330) and c) *Lentinula boryanus* (strain 402).

DISCUSSION

Edible mushrooms have demonstrated many medicinal properties; therefore, are strong candidates for potential nutraceutic agents. This study analyses *in vitro* nematocidal activity of mycelia from a group of edible mushrooms against *H. contortus* infective larvae. Other studies have shown that *P. ostreatus* produces a nematotoxin similar to peroxidases which inhibit the movement of nematodes and subsequently degrade them, reaching a mortality of 95% in the free-living nematode *Panagrellus redivivus* (adults) and the phytopathogen nematode *Bursaphelenchus xylophilus* [15].

The information obtained in the present study is similar to the data reported by these authors, since the mortality values obtained with *P. ostreatus* ECS-0152 and ECS-1123 were 92.1% and 97.2%, respectively. Another study conducted by [16], shows that *P. eryngii* attains 50% mortality against the phytopathogenic nematode *Heteroderas chantii* which causes wilting in sugarcane and other crops. Similar to our findings, these authors reported movement inhibition and nematode body invation and degradation. Interestingly, the mortality percentage obtained in the present study against *H. contortus* L_3 with *P. eryngii* ECS-1292 and ECS-1290 strains was higher (98.1 and 82.2%, respectively).

Table 2. Nematode-toxic edible mushrooms, nematicidal compounds, general conditions and efficacy.

Genus/specie of mushroom	Nematicidal compounds	Test nematodes	Efficacy	References
Pleurotus ostreatus	————	*Heterodera schachtii*	%	Palizi*et al*. [16]
Pleurotus ferulae	Cheimonophyllon E.5-hydroxymethyl-furancarbaldehyde.	*Bursaphelenchus xilophilus, Panagrellus redivivus*	—	Li *et al.* [17,18]
Pleurotus ostreatus	Trans-2-decenedioic acid.	*Panagrellus redivivus*	95	Kwok *et al.* [19]
Pleurotus pulmonarius	*p*-anisaldehydep-anisyl alcohol1-(4-methoxyphenyl) -1,2-propanediol.2-hydroxy-(4´-methoxy)-propiophenone. Fatty acid S-coriolic acid.	*Caenorhabditis elegans*	—	Stadler *et al*. [20] Koitabashi *et al*. [21]
Pleurotus ostreatus	————	*Meloiodgyne arenaria*	87-94	Xiand and Feng, [22]
Coprinus comatus	5-Methylfuran-3-carboxylic acid.5-hydroxy-3.5-dimethylfuran -2(5H)-one.5-hidroxy-3-(hydroxymethyl)-5-methylfuran -2(5H)-one4,6-dihydroxyiso benzufuran-1,3-dione4,6-dihydroxyisobenzufuran-3 (2H)-one4,6-dihydroxyiso benzufuran-1(3H)-one3-formyl-2.5-dihydroxybenzyl acetate	*Panagrellus redivivus, Meloidogyne javanica*	90	Luo *et al.* [13]

A list of nematode-toxic mushrooms, their nematocidal compounds and their nematocidal efficacy against genera/specie of different taxonomic groups of nematodes, are shown on Table 2. In general, nematocidal activity shown by the different genera/specie of assessed mushrooms ranged between 4.8-99.6% mortality.

The nematode lethal effect of specific edible mushroom, could be influenced by a number of factors, for example: temperature and incubation time of the confrontation or even inner genetic characteristics of each of the strains and/or differences between nematode species used. The fact that dead larvae that had not been invaded by any fungal mycelium were found suggests that as with *Coprinus comatus* strains, a nematotoxin can be produced by these mushroom species, resulting in nematode death. Alternatively, the fact that some dead larvae were observed with mycelium inside their bodies could

suggest that the larvae death was a consequence of body rupture and invasion by fungal mycelia. However, another possibility is that larvae could have been invaded by fungal mycelia after death. Currently, we do not know if the nematotoxin produced by these strains is sufficiently active to kill larvae before invading their bodies. However, Mamiya [13] found that a strain of *C. comatus* immobilizes, kills and consumes free-living nematodes *Panagrellus redivivus* and the root-knot nematode *Meloidogyne arenaria*. Authors of the present study, did not find any data regarding the lethal activity of *C. comatus* against *H. contortus* larvae; thus this study could be the first regarding this.

With respect to the strain *C. comatus*, Mamiya [13] reported mechanical damage in the free-living nematode *P. redivivus*, 8 h post-confrontation with *C. comatus* mycelia, resulting in 90% nematode immobilization and subsequent degradation, at 24 °C incubation. Data obtained in the present study differs from the results found by these authors, since *C. comatus* ECS-1103 caused 58.3% mortality at 5 days post-confrontation. These differences may be associated with the previously described factors; however, in both studies mechanical damage was observed in nematodes. In relation to *L. edodes*, Dong *et a*l. (2006), reported that the mortality against the phytonematode *B. xylophilus* was 57.6% after 72 h of incubation at 26 °C, while in the present study we found that *L. edodes* ECS-0401 presented 99.6% mortality against *H. contortus* (L_3) after 5 days of incubation at a temperature range of 18-25 °C.

Iijima *et al*. [7], isolated and cloned nematode degrading molecules (PCL-F) from the fungus *P. cornucopiae*, composed of two homodimers and a heterodimer combined with two subunits with 16 and 15 k Da molecular weight. These molecules PCL-F showed similarities with lectins from traps formed by the nematophagous fungus *Arthrobotrys oligospora*. Lectins are carbohydrate-binding proteins and their function is the recognition of molecules in the interaction of a variety of organisms, such as fungus-nematode. However, no information was found on the confrontation of edible mushroom mycelia against any nematodes at such level of complexity.

Regarding the strains *Panus* sp. ECS-801 and *Lentinula boryana* ECS-0402, no report about nematocidal activity of mycelia of these genera/specie was found. Thus, according to the results of the present research, both of these genera/specie of edible mushrooms have the potential for controlling sheep haemonchosis. To achieve this goal future research will be required.

CONCLUSIONS

The strains of the edible mushrooms *P. ostreatus* ECS-1123 and ECS-0152, *P. eryngii* ECS-1290 and ECS -1291, *P. cornucopiae* ECS-1328 and ECS-1330 and *L. edodes* ECS-0401 displayed high nematocidal activity, presenting a range of 82 to 99% mortality, while *Coprinus comatus* ECS-1103, *Panus* sp. ECS-801 and *L. boryana* ECS-0402 showed a low *in vitro* nematocidal activity against *H. contortus*, considered as the most economically and pathogenic parasite affecting the sheep industry worldwide.

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