EFFECT OF *Lentinula Edodes* SPENT MUSHROOM SUBSTRATE EXTRACTS ON *Meloidogyne incognita* EGGS HATCHING

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ABSTRACT

This study investigates the effect of aqueous and organic extracts of *Lentinula edodes* spent mushroom substrate (SMS) on the hatching of *Meloidogyne incognita* eggs. Samples of spent substrate were collected and processed with six different extraction methods. The resulting extracts were then assessed for their effect on *M. incognita* eggs *in vitro*. The findings reveal that acidic, alcoholic, and alkaline extracts of the SMS exhibited potential in controlling the hatching of *M. incognita* eggs. This study highlights the potential of *L. edodes* mushroom production waste as a source of extracts with ovicidal activity against nematode, offering a promising approach for establishing a sustainable process to obtain biomolecules for agricultural interest.

Keywords: Mushroom. Biocontrol. Shiitake. Root-knot nematode. Filamentous fungi.

1 INTRODUCTION

Fungi play a fundamental role in maintaining life on Earth. In addition to their benefits to the environment as decomposers of organic matter, they are also important sources of nutrition and bioactive metabolites. Due to the wide applicability of these microorganisms, they are expected to be included in circular bioeconomy models [1].

The production and consumption of mushrooms worldwide are increasing, with China being the largest producer globally. Although Brazil's production is still growing, it ranks among the five largest mushroom producers in the Americas, producing almost 16 million tons in 2018 [2]. In Brazil, the species *Agaricus bisporus*, *Lentinula edodes*, and *Pleurotus* spp. are mainly produced [3]. Shiitake, a *L. edodes* mushroom, is one of the most produced due to its composition rich in fiber, protein, and β-glucans [4].

The waste generated in the production of mushrooms, known as Spent Mushroom Substrate (SMS), is typically discarded into the environment, posing potential adverse effect. However, it can be incorporated into sustainable process models. The processing of this material can yield bioactive metabolites with diverse industrial applicability [5].

Potential beneficial effects on agriculture through the use of SMS have been reported. For instance, SMS water extract from *Lentinula edodes* was found to effectively control the conidia of *Pyricularia oryzae*, the fungus responsible for rice blast disease, with at least three compounds exhibiting fungicidal activity [6]. In another study, researchers observed a reduction in the reproduction of *Meloidogyne javanica* in lettuce following the incorporation of *Pleurotus djamor* SMS into the soil [7]. Furthermore, the nematicidal effect of *L. edodes* SMS water extracts have been observed against the nematode *Panagrellus* spp. [8], as well as *M. incognita* and *M. javanica* [9]. Also, extraction with different solvents can enhance the metabolites obtention, consequently improving the bioactive effect of SMS extracts. Therefore, establishing the process for obtaining extracts from SMS is crucial for obtaining bioactive stable molecules.

To further explore the potential applicability of SMSs in sustainable agriculture, this study aims to evaluate various methods of extracting SMS from *Lentinula edodes* to obtain extracts with ovicidal effects on *M. incognita*.

2 MATERIAL & METHODS

Lentinula edodes SMS was collected from three different fungi farmers located in the state of São Paulo, Brazil (designated as SMS_1, SMS_2, and SMS_3). The material was crushed and subjected to six different extraction solvents in a 3:7 (m/v) ratio:

i) Infusion in water without agitation at 55°C in a water bath,

ii) Infusion in water with agitation between 55 and 60°C for 2 hours,

iii) 70% alcohol,

iv) Aqueous solution acidified with 30% phosphoric acid (m/m),

v) Aqueous solution alkalinized with 30% potassium hydroxide (KOH), and

vi) Aqueous solution with 1% surfactant (siloxane) for 24 hours.

All extracts were subsequently filtered using Nº. 4 filter paper.

The *in vitro* ovicidal effect was evaluated with eggs of the root-knot nematode, *M. incognita*. For this, an aqueous suspension containing 400 eggs was exposed to 5% of each extract for a period of up to 14 days at 28 ºC. The hatched juveniles were counted in the Peters counting chamber. The assays were performed in triplicate. The control was obtained by incubating the same amount of eggs in water.

The data obtained were analyzed using ANOVA followed by a Tukey post-test using R software version 4.3.3.

3 RESULTS & DISCUSSION

Lentinula edodes SMSs demonstrated efficacy in controlling the hatching of *M. incognita*, with results suggesting a dependency on both the extraction method and the production location (**Figure 1**).

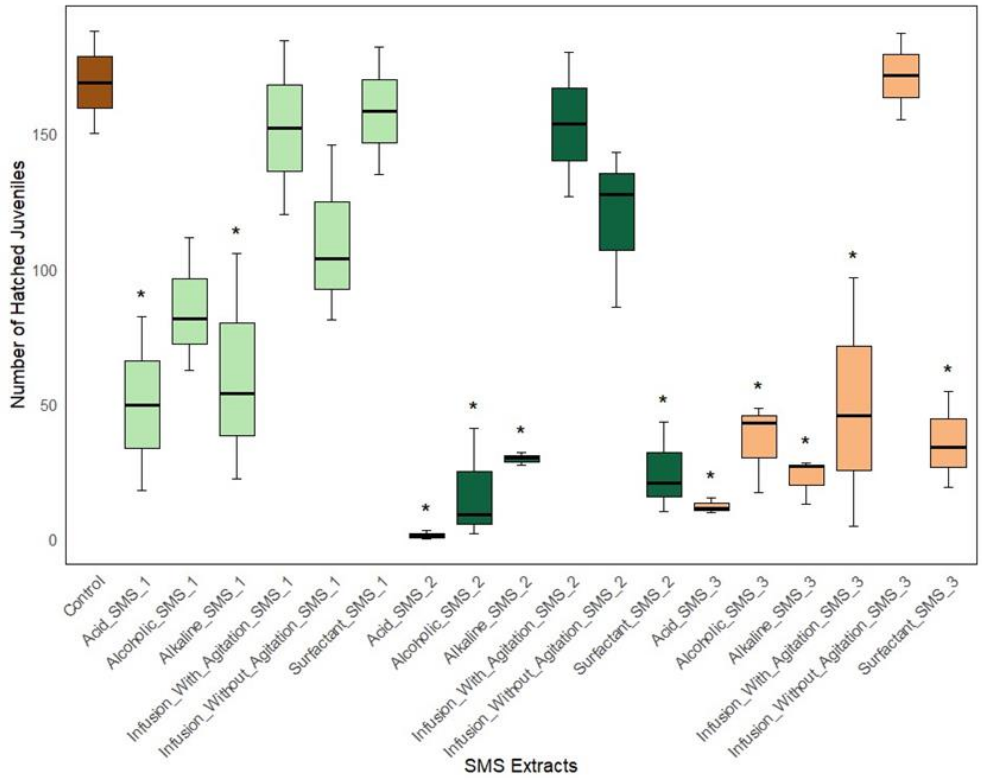

Figure 1 Number of hatched *M. incognita* eggs exposed to *L. edodes* SMS extracts. Boxplots marked with * indicate significant differences compared to the control, considering a significance level of $p < 0.01$.

Regardless of where the SMS was obtained, acid, alcoholic, and alkaline extractions showed the best effect in inhibiting the hatching of *M. incognita* eggs. Surfactant extraction exhibited a significant difference in hatching control only for the SMS sources SMS_2 and SMS_3. The different substrate preparation methods and conditions applied during mushroom fructification can interfere with the production of secondary metabolites [10,11], thereby influencing the control of *M. incognita* eggs hatching.

The extraction method is also a crucial factor in obtaining bioactive metabolites [12]. For example, the water infusion method, which did not show a statistical difference compared to the control in this study, has been demonstrated in other research to be efficient in extracting metabolites using SMS from *L. edodes* [6,9]. The presence of components in alcoholic, acidic, alkaline, and surfactant extractions interferes with the pH and osmolarity of the mycelium cell wall, enabling the extraction of metabolites with different characteristics [13]. These components proved to be essential for controlling the hatching of *M. incognita* in the present work.

The results obtained in this study demonstrate the potential applicability of waste generated from Shiitake mushroom production in obtaining extracts suitable for integration into a sustainable circular economy model. After the cultivation of mushrooms for human consumption, the resulting waste can be repurposed to extract metabolites of agricultural interest. However, further *in situ* studies are necessary to evaluate the direct effects of these extracts in field conditions.

4 CONCLUSION

In conclusion, the acid, alcoholic, and alkaline extracts of *L. edodes* SMS obtained from different farmers demonstrate potential in controlling, *in vitro*, the hatching of *M. incognita* eggs.

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