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STRAINS OF *LENTINULA EDODES* SUPPRESS GROWTH OF PHYTOPATHOGENIC FUNGI AND INHIBIT ALAGOAS SEROTYPE OF VESICULAR STOMATITIS VIRUS

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ABSTRACT

Four *Lentinula edodes* strains (Le10, 46, K2, Assai) were assessed for their antagonistic effect on four filamentous fungus species of agricultural importance (*Helminthosporium euphorbiae*, *Helminthosporium* sp, *Fusarium solani and Phomopsis sojae*) and on Alagoas serotype of Vesicular Stomatitis Virus (VSA). The *L. edodes* strains studied had variable effects on the filamentous fungi and on VSA. The K2 and Le10 strains were antagonistic on the fungi assessed and the 46 and K2 strains were efficient on the Vesicular Stomatitis Virus. The results widened the list of beneficial effects of *L. edodes* on the control and prevention of animal pathogenic virus and filamentous fungi.

Key words: Phytopathogenic fungi, shiitake, inhibition, Vesicular Stomatitis Virus (Alagoas)

INTRODUCTION

Lentinula edodes is an edible mushroom, which has been cultivated in the Asian countries for centuries. Lately its cultivation was extended to several areas of Brazil, and it has been employed in human feeding. It is known as 'hoang-mo' in China while its commercial name in Japan is 'shiitake'(5). The ancients considered it the elixir of life because of its high nutritional content (3). The therapeutic importance of *L. edodes* has been known since the Ming dynasty (1368-1644) from reports that shiitake increased the vigor and energy in humans (6). Several papers in the literature reported the production of substances with therapeutic and medicinal properties (including antibiotic, antiviral, antitumors and antithrombosis) isolated from the fruit body, spores, mycelia and mycelial culture medium of *L. edodes* (7,9,17). *L. edodes* has shown activity in mice against VSV encephalitis (Vesicular Stomatitis Virus) and Abelson virus with 100% cure (17). Extracts of the fruiting bodies of *L. edodes* have been shown to contain also potent inhibitors of plant virus. This purified inhibitor was found to be a basic simple protein without carbohydrate content (8). The purpose of the present study was to examine the possibility of the use of shiitake extracts as a preventive agent against filamentous fungi of agricultural interest and on Vesicular Stomatitis Virus – Alagoas (VSA).

MATERIALS AND METHODS

Lentinula edodes strains

Four strains of *L. edodes* were used, Le10, 46, Assaí and K2, from the stock of fungi at the State University of Londrina/ PR, Brazil.

Phytopathogenic fungi strains

The antagonistic effect of *L. edodes* strains was assessed on the filamentous fungi, *Helminthosporium euphorbiae*, *Helminthosporium* sp., *Fusarium solani* and *Phomopsis sojae* obtained from the stock of fungi of the Phytopathology Laboratory of Embrapa Soya at Londrina/PR, Brazil.

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Virus

Alagoas serotype of Vesicular Stomatitis Virus (VSA) was obtained from the collection of the Department of Microbiology at the State University of Londrina/PR, Brazil.

Extract from *L. edodes* mycelium and culture filtrate

The *L. edodes* strains were separately grown on potato-dextrose-agar medium, at 25ºC in the dark. An agar disk (10 mm diameter) bearing mycelium of *L. edodes* was placed in 100 mL of potato-broth at 25ºC for 20 days. The cultures were filtered vacuum in a Buchner filter and the filtrate, denominated Culture Filtrate (CF), was sterilized in Millipore membrane (0.22 µ) and stored at 4ºC until use. The mycelia were squashed in sterilized distilled water (ratio 0.5g : 4 mL water) and centrifuged at 3,000 rpm for 10 minutes. The supernatant, denominated Mycelial Extract (ME), was stored at 4ºC.

Assessment of *L. edodes* effect on filamentous fungi

An agar disc (10mm diameter) bearing mycelium of each phytopathogenic fungi was placed on the surface of PDA medium (20 mL) (potato dextrose agar- Biobrás) added of 10%, 20% and 30% of CF of *L. edodes* and incubated at 28ºC in the dark. After the second day of incubation, daily measurements of the diameter (mm) of colony growth were taken and recorded. The experiments were carried out in quadruplicate. These values were converted into area and corrected by the formula below to compare the treatments:

$$CV = 1 + \frac{FMAC - IA}{FMAC - IAC}$$

Where:
CV = Corrected Value;
IA = Inoculum Area;
FMAC = Final Means Area of the Control;
IAC = Inoculum Area of the Control.

Assessment of *L. edodes* effect on VSA

Vero cells were cultivated in 96-well tissue culture microplates for 48h in medium 199 supplemented with F10 (Sigma) at 37ºC in 5% CO_2. The culture media were substituted by fresh media containing virus at 10^{-1} to 10^{-7} dilutions with or without ME and CF from four *L. edodes* strains. ME and CF dilutions (1:10, 1:20, 1:30, 1:40, and 1:80) were previously tested for cytotoxic effects on cell culture. VSA - infected and noninfected cell cultures treated with *L. edodes* ME and CF were maintained for control. The experiments were carried out in triplicate. Cell cultures were daily observated and viral titer (titer capable of infecting 50% of the cell cultures per mL - $TCID_{50}$/mL) was calculated according to Reed and Muench (12). The viral inhibition percentage was calculated as described in Nishimura *et al.* (10).

RESULTS AND DISCUSSION

Assessment of *L. edodes* effect on phytopathogenic fungi

The results showed that *L. edodes* strains, K2 and Le10, had a greater inhibitory effect on the phytopathogenic fungi than the 46 and Assaí strains (Table 1).

The 0.3 mL *L. edodes* culture filtrate dose had the greatest antagonistic effect on the fungi, regardless the *L. edodes* strain used (Table 2).

The *H. euphorbia* and *Helminthosporium* sp fungi were more susceptible to the *L. edodes* treatment than the other fungi (Table 3).

The filamentous fungi assessed in this work infect mainly the soybean (*Glycine max*) crops decreasing yield. *H. euphorbiae*, however, is a phytopathogen with great potential

Table 1. Antagonism of four *Lentinula edodes* strains on phytopathogenic fungi (*Phomopsis sojae*, *Fusarium solani*, *Helminthosporium* sp., *Helminthosporium euphorbiae*).

Area means (mm) *	*L. edodes* strains
17,836 a	Assaí
17,757 a	46
15,914 b	Le10
14,675 b	K2

* Means represented by the same letter do not differ significantly by the Tukey test ($p < 0.05$).

Table 2. Effect of different *Lentinula edodes* culture filtrate doses on the growth of the phytopathogenic fungi.

Area means (mm)*	Doses
17,390 a	D1
16,924 a	D2
15,323 b	D3

* Means represented by the same letter do not differ significantly by the Tukey test ($p < 0.05$). D1 = 0.1 mL *L. edodes* CF from PDA medium; D2 = 0.2 mL *L. edodes* CF from PDA medium; D3 = 0.3 mL *L. edodes* CF from PDA medium.

Table 3. Effect of the culture filtrate of *Lentinula edodes* on the growth of the phytopathogenic fungi.

Area means (mm)*	Fungi
18,496 a	*Phomopsis sojae*
18,046 a	*Fusarium solani*
14,501 b	*Helminthosporium sp*
11,00 c	*Helminthosporium euphorbiae*

* Means represented by the same letter do not differ significantly by the Tukey test ($p < 0.05$).

for use in biological control of the weed *Euphorbia heterophpylla*, known popularly as Moleplant in soybean crops (14,15). *Helminthosporium* sp. affects many grasses (wheat, rye, etc.) causing localized lesions and root rotting (4). *Fusarium solani* causes the red root rot disease and it is a problem in soybean seed production (16). *P. sojae* is the pathogen that causes the dry or burn stem disease, which is very common in soybean crops (4). These diseases are normally controlled with chemicals and adequate soil management. The use of natural products for the control of these diseases is a promising alternative and has received much attention worldwide.

Pacumbaba *et al.* (11) determined the inhibitory activity of the mycelial leachate of *L. edodes* on the growth of several important species of plant pathogenic bacteria in the laboratory. The mycelial leachate applied as soil drench prevented symptom expression of soyborn bacterial pathogens, suggesting that the shiitake mycelial leachate contains an antibiotic component.

In the literature, the effect of *L. edodes* on fungi is described only in the yeast *Candida albicans* and a compound, denominated Cortinellin, isolated from *L. edodes* showed antibiotic activity against this yeast (1). Therefore, this is the first time that mycelial extract of *L. edodes* has been reported to inhibit the growth of phytopathogenic fungi.

Table 4. Effect of the interaction of the culture filtrate and the mycelial extract of four *Lentinula edodes* strains in the replication of VSA in Vero cell culture, in $TCID_{50}$/mL.

L. edodes Strains*		Treatment**	Control**
K2	ME	$10^{3,24}$	$10^{5,24}$
	CF	$10^{5,24}$	$10^{5,24}$
Le10	ME	$10^{5,24}$	$10^{5,24}$
	CF	$10^{5,24}$	$10^{5,24}$
46	ME	$10^{4,24}$	$10^{5,24}$
	CF	$10^{3,24}$	$10^{5,24}$
Assaí	ME	$10^{5,24}$	$10^{5,24}$
	CF	$10^{5,24}$	$10^{5,24}$

* ME: Mycelial Extract; CF: Culture Filtrate; ** Viral titer expressed in $TCID_{50}$/ mL and calculated according to Reed and Müench (1938).

Table 5. VSA inhibition percentage caused by four *Lentinula edodes* strains in Vero cell culture.

L. edodes Strains	ME *	CF **
K2	99%	0
46	90%	99%
Assaí	0	0
Le10	0	0

* ME: Mycelial Extract; **CF: Culture Filtrate

***L. edodes* effect on VSA**

The ME and CF did not have a toxic effect on the Vero cell culture at the 1:20 (Le10 and Assaí strains) or 1:30 dilutions (K2 and 46 strains). Table 4 shows the result of the ME and CF interaction and the cytopathic effect of the virus.

The percentage of viral inhibition was calculated for each *L. edodes* strain (Table 5) based on viral titer in the presence or absence of ME and CF.

Only two *L. edodes* strains were effective in inhibiting VSA replication. Strain 46 was an effective antiviral both with ME and CF. The latter was more efficient with 99% inhibition. The K2 strain was efficient only with ME, showing an inhibition percentage of 99%.

The VSA causes a disease characterized by the appearance of vesicular lesions in the mouth and hooves of horses but it can also affect swine and cattle. In swine lesions may lead to the loss of the hoof. Mouth lesions (which are the most important) are not usually treated because they heal naturally within a week. The disease causes weight loss in cattle because of the temporary inability to eat, and a decrease in milk production of around 20%, which returns to 70-80% of the normal production within a month. The symptoms of this infection are very similar to foot and mouth fever and the swine vesicular disease and thus deserve attention (2).

Tsunoda and Ishida (13) described indirect *L. edodes* antiviral activity in watery extract from the fruit body and spores. As described in the present study, the authors observed that the extract from the shiitake fruit body contains potent plant virus inhibitors. Kobayashi *et al.* (8) also described a substance from the *L. edodes* fruit body that had inhibitory action on the plant virus infection. This substance was described as being a protein. Various substances from the mycelia and *L. edodes* fruit body have already been reported as having antiviral activities in plants. Tests of these substances used as an antiviral have been made for the inhibition of viral infection in plants (8).

The present results widen the list of beneficial effects of *L. edodes* and suggest the possibility of using this basidiomycete in the control and prevention of other animal viral and fungal infections of agronomic and veterinary interest. But the data also showed a variability among the strains studied for the assessed traits were, not all the strains had an inhibitory effect on the VSA virus and on the fungi. So, the generalization of the antifungal and antiviral effect for the *L. edodes* species as a whole should be made with caution.

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RESUMO

Linhagens de *Lentinula edodes* inibem fungos fitopatogênicos e o vírus da estomatite vesicular, sorotipo Alagoas

Quatro linhagens de *Lentinula edodes* (Le10, 46, K2, ASSAI) foram avaliadas quanto ao seu efeito inibitório sobre quatro espécies de fungos filamentosos de importância agrícola (*Helminthosporium euphorbiae, Helminthosporium* sp., *Fusarium solani*, *Phomopsis sojae*) e sobre o sorotipo Alagoas vírus da estomatite vesicular (VSA). Foi observado que as linhagens de *L. edodes* estudadas apresentaram variabilidade quanto ao seu efeito, tanto sobre os fungos filamentosos quanto sobre o vírus VSA. As linhagens K2 e Le10 apresentaram-se antagônicas sobre os fungos e as linhagens 46 e K2 foram eficientes na inibição do vírus VSA. Os resultados obtidos permitem ampliar a lista de efeitos benéficos de algumas linhagens de *L. edodes* no controle e prevenção de vírus patogênicos animais e de fungos filamentosos.

Palavras-chave: Fungos fitopatogênicos , shiitake, inibição, vírus VSA

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