

## EXTRATO AQUOSO DE RESÍDUO DO PROCESSAMENTO DE *Lentinula edodes* NO CONTROLE DE FUNGOS FITOPATOGÊNICOS

NICOLAS AUGUSTO DE CARVALHO FRANCO<sup>1</sup>, MIRELA MARIA MAGANHA<sup>2</sup>, OLÍVIA GOMES MARTINS<sup>3</sup>, LISANDRO DE PROENÇA PIERONI<sup>4</sup> E MEIRE CRISTINA ANDRADE CASSIMIRO DA SILVA<sup>5</sup>

1 Graduando em Engenharia Agrônômica, Faculdade Galileu. Av. Marginal 200, 680 - Vila Real, Botucatu - SP, 18606-294, Botucatu, SP, Brasil. E-mail: nicolas.augusto.07@gmail.com

2 Graduada em Engenharia Agrônômica, Faculdade Gran Tietê. Av. 15 de Novembro - Centro, Barra Bonita - São Paulo, 17340-000, Barra Bonita, SP, Brasil. E-mail: mirelamaganha@gmail.com

3 Doutora em Agronomia – Energia na Agricultura, Faculdade de Ciências Agrônômicas, Universidade Estadual Paulista "Julio de Mesquita Filho" (UNESP), Av. Universitária, 3780 - Altos do Paraíso, 18610-034, Botucatu, SP, Brasil. E-mail: oliviagmartins@gmail.com

4 Doutorando em Agronomia – Ciência Florestal, Faculdade de Ciências Agrônômicas, Universidade Estadual Paulista "Julio de Mesquita Filho" (UNESP), Av. Universitária, 3780 - Altos do Paraíso, 18610-034, Botucatu, SP, Brasil. E-mail: lisandro.pieroni@unesp.br

5 Docente da Faculdade Galileu. Av. Marginal 200, 680 - Vila Real, Botucatu - SP, 18606-294, Botucatu, SP, Brasil. E-mail: mcnandrade@hotmail.com

**RESUMO:** Um dos principais custos do agronegócio é os de insumos utilizados no controle fitossanitário, principalmente no controle de doenças. A demanda do agronegócio por soluções sustentáveis faz com que a busca por insumos alternativos seja constante uma vez que, principalmente pequenos produtores, podem se beneficiar destes produtos. Entre essas alternativas está o uso de extratos aquosos fúngicos, do qual muitos possuem substâncias com propriedades antagônicas contra fitopatógenos. O objetivo desse trabalho foi testar a eficiência do extrato aquoso de resíduo do processamento de *Lentinula edodes* no controle dos fitopatógenos *Corynespora* sp. (pepino) e *Fusarium* sp. (alface). A obtenção dos extratos foi realizada com os basidiomas desidratados em pó, misturados em água destilada e incubados a 4°C por 18 h. Após o período de incubação, a suspensão foi filtrada, centrifugada e o sobrenadante filtrado em membrana tipo Millipore (diâmetro do poro = 0,2 µm). O extrato obtido foi incorporado ao meio de cultivo B.D.A, nas proporções de 0; 1; 2; 3 e 4% (v/v) e no centro de cada placa foi adicionado um disco de micélio de cada fitopatógeno. O delineamento experimental foi em esquema fatorial 2 x 5 (2 fitopatógenos e 5 concentrações), inteiramente casualizado, com 6 repetições. O crescimento micelial foi quantificado e os dados obtidos foram submetidos à análise de variância e as médias comparadas pelo teste de Turkey (5%). De acordo com os resultados o extrato aquoso de *L. edodes* possui potencial no controle biológico destes fungos, sobretudo de *Fusarium* sp., em concentrações acima de 2%.

**Palavras-chave:** controle alternativo, extrato natural, sustentabilidade.

## AQUEOUS EXTRACT OF RESIDUE FROM THE PROCESSING OF *Lentinula edodes* IN THE CONTROL OF PHYTOPATOGENIC FUNGI

**ABSTRACT:** One of the main costs of agribusiness are the inputs used in phytosanitary control, mainly in disease control. Agribusiness requires alternative solutions with the search for constant inputs, since, mainly, producers can be beneficiaries of these products. Among alternatives is using these aqueous extracts, none of them have substances with antagonistic properties, the objective of this work was to test the efficiency of the aqueous extract of adjustment of these treatments of *Lentinula edodes* processing and of the processing of fungi, without control of the phytopathogens, *Corynespora* sp. (cucumber) and *Fusarium* sp. (lettuce). The extraction was carried out with the basidiomes dehydrated in powder, mixed in distilled water and incubated at 4°C for 18°C. After the incubation period, the suspension was filtered, centrifuged and the supernatant type filtered through a Millipore membrane (pore diameter = 0.2µm). The extract obtained was incorporated into the

culture medium B.D.A, in the proportions of 0; 1; two; 3 and 4% (v/v) and in the center of each plate a disc of phytopathogenic mycelium was added. The experimental design was in a 2 x 5 factorial scheme (2 fit wasopathogens and 5 were performed), with 6 completely randomized replications. Growth mice and data were selected for analysis of analyzes of analyzes produced by the variance test (5%). According to the results, the aqueous extract of *L. e* has potential to control these biologicals, especially *Fusarium sp.*, in which it is above 2%.

**Keywords:** alternative control, natural extract, sustainability.

## 1 INTRODUCTION

To meet the growing global demand for food, modern agriculture has made several advances in different areas, such as mechanization, variety improvement, and the synthesis of more effective chemical molecules for pest and disease control.

From a phytosanitary point of view, the control of biological diseases is a decisive factor in agricultural production and is extremely important depending on the plant species involved, the year of cultivation and cultural management.

Plants possess a dynamic defense system with diverse abilities that act when they sense the presence of invaders and the ability to inhibit, delay, or exclude pathogens from their tissues. However, some plants lack a system to suppress certain diseases that may eventually develop, which can affect their vigor if there are no other effective means of control (STANGARLIN). *et al.*, 2011). However, other control methods can help prevent and control plant-invading diseases, and in addition to other related chemical methods, care must be taken to avoid induced resistance in each host species.

According to Di Piero (2003), induced resistance is a general term that defines a process of resistance activity that depends on the physical and/or chemical barriers of the host plant, which are activated by biotic or abiotic agents, the so-called inducers.

In the search for natural source controls and the analysis of the various uses of fungi, specifically mushrooms, through a survey of some of their characteristics, they can be presented as viable and sustainable alternatives since they possess properties that qualify as medicinal (MAHAJNA *et al.*, 2009), which can inhibit and control some types of fungi, and the production of some species can be rustic and

quick, with the reuse of dry materials and agricultural waste (SAAD *et al.*, 2017).

When the properties of mushrooms are analyzed briefly, as Eira *et al.* (2005) reported, they possess nutraceutical properties that are protective to the organism. Furthermore, by observing the characteristics of some economically important diseases in both lettuce and cucumber, it is possible to develop a more efficient and sustainable management plan to control these diseases, reducing the use of agrochemicals and replacing them with natural extracts. This will consequently decrease a significant number of chemical residues in these vegetables, delivering a more natural product to the end consumer and protecting the environment.

Given this context, this research aimed to evaluate the efficiency of an aqueous extract of *Lentinula edodes processing residue* in controlling phytopathogenic fungi in lettuce and cucumber.

## 2 MATERIALS AND METHODS

### 2.1 Preparation of the aqueous extract

*Lentinula edodes* residues were obtained according to the methodology of (PICCININ; PIERO; PASCHOLATI, 2010), with adaptations, and are described below. Initially, the basidiomes of *L. edodes* were dehydrated into powder, mixed in distilled water (14 mL/cm<sup>2</sup>) and incubated at 4°C for 18 h. After the incubation period, the suspension was filtered through a common filter (8 g cm<sup>-2</sup>), which was subsequently centrifuged at 15,557 g for 20 min<sup>-1</sup> at 15°C. After centrifugation, the supernatant was filtered through a Millipore membrane (pore diameter = 0.2 µm), and the crude aqueous extracts were stored at 4°C for later use in *in vitro tests*.

## 2.2 In vitro tests

The effects of the aqueous extract (AE) on the mycelial growth of the phytopathogens *Corynespora* sp. (Cucumber) and *Fusarium* sp. (Lettuce) were tested via the following methods. The aqueous extract was incorporated directly into potato dextrose agar (PDA) culture media at proportions of 0, 1, 2, 3, and 4% (v/v) and poured into Petri dishes. After three days, a mycelial disc of each phytopathogen (0.5 cm in diameter) was added to the center of the plates, which were subsequently incubated at 25°C in the dark. The growth of the fungal colonies was quantified when the mycelial growth of replicate 17, corresponding to one of the plates in treatment 3, reached the maximum diameter of the Petri dish, approximately 7.66 cm, on the seventh day of monitoring, corresponding to the fungus *Corynespora*.

## 2.3 Experimental design

The experimental design was a  $2 \times 5$  factorial scheme (2 phytopathogens and 5

concentrations), which was completely randomized, with 6 repetitions, totaling 60 experimental units (Petri dishes).

## 2.4 Statistical analysis

The data obtained were subjected to analysis of variance, and the means were compared via Tukey's test (5%) (SNEDECOR; COCHRAN, 1972). The analysis was performed via the SISVAR 4.2 program, which was developed by the Department of Exact Sciences, Federal University of Lavras, MG (UFLA).

## 3 RESULTS AND DISCUSSION

The growth of the fungi can be seen in Graph 1, which shows the development in millimeters and the fungi over seven days. *Corynespora* sp. grows, whereas *Fusarium* sp. reacts to the extracts from the fourth day onward and is considered, on the basis of the results, to be more susceptible to the aqueous extract of *L. edodes*.

**Figure 1** Fungi over time.

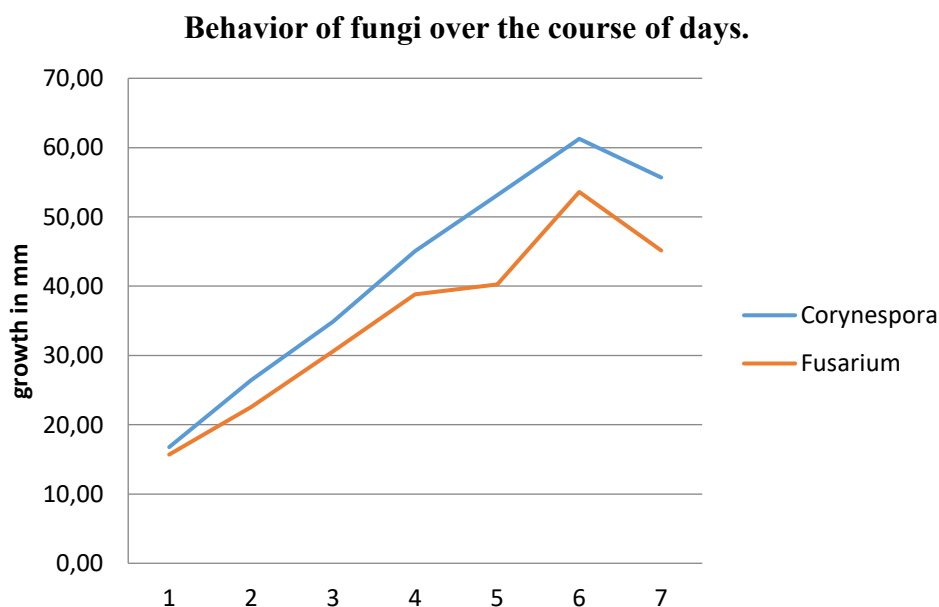


Table 1 shows the average growth in millimeters of the fungi in relation to the concentrations of the aqueous extract, where means followed by the same letter do not differ from each other according to Tukey's test ( $p >$

0.05), which was the baseline test in this study. That is, there was greater fungal development at concentration 0 (control), and it was statistically equal to that at concentration 1; however, it differed from the other

concentrations. Concentration 1 did not differ from any of the other concentrations;

concentrations 2, 3 and 4 did not differ from 1 but did differ from 0.

**Table 1** Average fungal growth in relation to concentration.

Concentrations	Average*
0	43,101 a
1	38,989 ac
3	37,497 bc
4	37,176 bc
2	36,873 bc

Concentration 0: control; Concentration 1: 1% basidioma in the aqueous extract; Concentration 2: 2% basidioma in the aqueous extract; Concentration 3: 3% basidioma in the aqueous extract; Concentration 4: 4% basidioma in the aqueous extract. Means followed by the same letter do not differ statistically according to Tukey's test at the 5% significance level.

Statistical analysis was performed considering the concentration of the aqueous extract in relation to each treatment, regardless of the fungus in question, to verify whether the concentration influences growth.

Table 2 presents the average final growth of the fungi and the significant difference during the test period, where *Corynespora* sp. reached an average of 41.86 mm and *Fusarium* sp. 35.60 mm; thus, *Fusarium* sp. was more susceptible to aqueous extracts. In an *in vitro* study by Figueiredo and

Castro e Silva (2014), an alternative control for *Fusarium* sp. was developed using aqueous and hydroalcoholic extracts of *Pycnoporus sanguineus* and *Lentinus crinitus*, where the cold hydroalcoholic extracts obtained the best results, whereas the aqueous extracts did not have relevant results. Thus, the aqueous extract of *L. edodes* is more efficient in the *in vitro* control of *Fusarium* sp. than the aqueous extracts of *Pycnoporus sanguineus* or *Lentinus crinitus* are.

**Table 2.** Average final growth of the fungi.

Fungi	Average*
<i>Corynespora</i>	41,846 a
<i>Fusarium</i>	35,609 b

Using the same methodology for preparing the aqueous extract of *L. edodes*, Toffano *et al.* (2012) tested its application on postharvest fungi affecting citrus; however, they did not find results that inhibited, eradicated, or cured the diseased fruit. In this case, it is possible to reinforce the ability of *L. edodes* to reduce the development of *Corynespora* sp. and *Fusarium* sp., with better inhibition of *Fusarium* sp.

In another study, aiming to verify the effectiveness of an alternative fungal extract for

the inhibition of phytopathogens in vegetables, Figueiredo (2012) used aqueous (hot, cold and ultrasonic) and hydroalcoholic (hot and cold) extracts of the basidiomycetes *Pycnoporus sanguineus* and *Lentinus crinitus* to control *Fusarium* sp. In these *in vitro* tests, all the extracts used yielded positive results; however, the ability of both aqueous and hot hydroalcoholic extracts to eradicate *Fusarium* sp. should be highlighted, given that heat can assist in the phytopathogen inhibition process.

#### 4 CONCLUSION

The aqueous extract of *L. edodes* has potential in the biological control of the fungi *Corynespora* sp. and *Fusarium* sp., especially *Fusarium* sp., at concentrations above 2%.

#### 5 REFERENCES

DI PIERO, RM. **Potential of *Lentinula edodes* (Shiitake) and *Agaricus blazei* (sun mushroom) mushrooms in the control of diseases in cucumber, passion fruit and tomato plants, and the partial purification of biologically active compounds.** 2003. Doctoral thesis (PhD in Phytopathology) – Luiz de Queiroz Higher School of Agriculture, University of São Paulo, Piracicaba, 2003. DOI: 10.11606/T.11.2003.tde-09122003-110305. Available at: <https://teses.usp.br/teses/disponiveis/11/11135/tde-09122003-110305/pt-br.php>. Accessed on: January 10, 2023.

EIRA, AF; NASCIMENTO, JS; COLAUTO, NB; CELSO, PG. Cultivation technology of the medicinal mushroom *Agaricus blazei* (*Agaricus brasiliensis*). **Agropecuária Catarinense** . Florianópolis, v. 18, n. 3, p. 45-49, Nov. 2005. Available at: [https://www.researchgate.net/profile/Nelson-Colauto/publication/262687308\\_Tecnologia\\_de\\_cultivo\\_do\\_cogumelo\\_medicinal\\_Agaricus\\_blazei\\_Agaricus\\_brasiliensis/links/0a85e538774039ff7b000000/Tecnologia-de-cultivo-do-cogumelo-medicinal-Agaricus-blazei-Agaricus-brasiliensis.pdf](https://www.researchgate.net/profile/Nelson-Colauto/publication/262687308_Tecnologia_de_cultivo_do_cogumelo_medicinal_Agaricus_blazei_Agaricus_brasiliensis/links/0a85e538774039ff7b000000/Tecnologia-de-cultivo-do-cogumelo-medicinal-Agaricus-blazei-Agaricus-brasiliensis.pdf) . Accessed on: Mar. 8, 2023. 2022.

FIGUEIREDO, AS. **"In vitro" evaluation of basidiomycete extracts against phytopathogens harmful to vegetable production by small producers in the lower Amazon region** . 2012. Dissertation (Master's in Biotechnology and Natural Resources) – State University of Amazonas, Parintins, 2012.

FIGUEIREDO, A.; CASTRO E SILVA, A. *In vitro* activity of extracts of *Pycnoporus sanguineus* and *Lentinus crinitus* on the phytopathogen *Fusarium* sp. **Acta Amazonica** , Manaus, v. 44, n. 1, p. 1-8, 2014.

MAHAJNA, JDN; DOTAN, N.; ZAIDMAN, BZ; PETROVA, RD; WASSER, SP. Pharmacological values of medicinal mushrooms for prostate cancer therapy: the case of *Gonoderma lucidum* . **Nutr Cancer** , Philadelphia, v. 61 n. 1 p. 16-26, 2009.

PICCININ, E.; PIERO, RM di; PASCHOLATI, SF. Shiitake mushroom (*Lentinula edodes*) reduces the growth of phytopathogens and the severity of leaf spots in sorghum. **Summa Phytopathol** , Botucatu, v. 36, n. 1, p. 68-72, Jan. 2010.

SAAD, ALM; VIANA, SRF; SIQUEIRA, OAPA; CAMPOS, CS; ANDRADE, MCN. Utilization of agricultural waste in the cultivation of the medicinal mushroom *Ganoderma lucidum* using the Chinese “Jun-Cao” technology. **Ambiência** , Guarapuava, v. 13, n. 3, p. 271-283, Jun. 2017. Available at: <https://revistas.unicentro.br/index.php/ambiencia/article/view/3643/pdf>. Accessed on: Mar. 14, 2022.

SNEDECOR, GWE; COCHRAN, WG. **Statistical methods** . 6. ed. Ames: Iowa State University Press, 1972.

STANGARLIN, JR; KUHN, OJ; TOLEDO, MV; PORTZ, RL; SCHWAN-ESTRADA, KRF; PASCHOLATI, SF. Plant defense against phytopathogens. **Scientia Agraria Paranaensis** , Cascavel, v. 10, n. 1, p. 18-46, Jan. 2011.

TOFFANO, L.; FISCHER, IH; BLUMER, S.; PASCHOLATI, SF. Potential of the flavedo (epicarp) of *Citrus aurantifolia* cv. Tahiti in the control of green mold and anthracnose in citrus. **Summa Phytopathologica** , Botucatu, v. 38, n. 1, p. 61-66, 2012.