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Tecnologias Limpas

Mushroom extract induces resistance in passion fruit and *in vitro* control of *Xanthomonas axonopodis* pv. *passiflorae*

Extrato de cogumelo na indução de resistência do maracujazeiro e controle in vitro de Xanthomonas axonopodis pv. passiflorae

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ABSTRACT: *In vitro* inhibitory capacity of *Lentinula edodes* and *Agaricus blazei* extracts on *Xanthomonas axonopodis* pv. *passiflorae* and their capacity to induce resistance in passion fruit in a greenhouse are evaluated. Aqueous crude extracts (CEs) were prepared by hydrating the basidiocarp dry powder with distilled water for 24h, at 4 ºC. CEs were added in test tubes at concentrations 10, 20, 30 and 40%, whilst control contained water only. Bacterial suspension (1 mL) was added to each tube (10^8 cfu.mL^{-1}) and incubated in the dark at 28 °C for 24h. The bacterial suspension was measured by a spectrophotometer at 550 nm. Passion fruit cultivars IAC-275 and Epagri Oval Grande were sown on two substrates in a greenhouse: substrate 1 (SB) - horizon B soil; substrate 2 (SBC) - 40% soil from horizon B + 40% organic compost + 20% roughage (rice husk). *A. blazei* aqueous crude extract (ACE) treatments were applied at 20% and 40%; *L. edodes* ACE 20% and 40%; biofertilizer Agromos® 1% and control (without treatment) were started when the plants were in the 4-6 leaf stage. Sprays were done weekly, totaling four applications. Seven days after the first application and seven days after the last spraying, leaves were collected to evaluate chitinase, peroxidase and β-1,3-glucanase activity seven days. *In vitro* aqueous mushroom extracts inhibited the CFU of *X. axonopodis* pv. *passiflorae* and increase in the activity of peroxidase and β-1,3-glucanase was observed in passion fruit plants in the greenhouse.

Keywords: *Agaricus blazei.* Bacterial spot. Chitinase. *Lentinula edodes*.

RESUMO: A capacidade inibitória, *in vitro*, dos extratos *Lentinula edodes* e *Agaricus blazei* sobre *Xanthomonas axonopodis* pv. *passiflorae* e seu potencial na indução de resistência do maracujazeiro; foi avaliada em casa de vegetação. Os extratos brutos aquosos (EBs) foram preparados a partir da hidratação do pó seco do basidiocarpo com água destilada por 24h, a 4 ºC. Os EBs foram adicionados em tubos de ensaio nas concentrações 10, 20, 30 e 40%, enquanto a testemunha continha somente água. A suspensão bacteriana (1 ml) foi adicionada em cada tubo (10^8 ufc mL^{-1}) e incubados no escuro a 28 ºC por 24h. A suspensão bacteriana foi aferida em espectrofotômetro em 550 nm. As cultivares de maracujazeiro IAC-275 e Epagri Oval Grande foram semeadas em dois substratos: substrato 1 (SB) - solo horizonte B; substrato 2 (SBC) - 40% de solo horizonte B + 40% composto orgânico + 20% de volumoso (casca de arroz). Os tratamentos extrato bruto aquoso (EBA) de *A. blazei* 20% e 40%; *L. edodes* (EBA) 20% e 40%; biofertilizante Agromos® 1% e controle (sem tratamento) foram aplicados quando as plantas estavam no estádio de 4-6 folhas. As pulverizações foram feitas semanalmente, totalizando 4 aplicações. Sete dias depois da primeira aplicação e sete dia depois da última pulverização, folhas foram coletadas para avaliação da ativação de quitinase, peroxidase e β-1,3-glucanase. Os extratos aquosos dos cogumelos inibiram, *in vitro*, as UFC de *X. axonopodis* pv. *passiflorae* e o aumento da atividade de peroxidase e β-1,3-glucanase foi observada em plantas de maracujá em casa de vegetação.

Palavras-chave: *Agaricus blazei. Lentinula edodes.* Mancha bacteriana. Quitinase.

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INTRODUCTION

Passion fruit is greatly significant in Brazilian fruit growing, especially for family farmers. Brazil is currently the world's largest producer of yellow passion fruit, with a production of 593,429 t (2019) and a cultivated area of 41,584 ha. It is a growing market based on the commercialization of fresh fruit, juice and pulp (IBGE, 2019).

However, phytosanitary problems have reduced the useful life of new crop-growing areas. One of the main problems is the passion fruit´s bacteriosis caused by *Xanthomonas axonopodis* pv. *passiflorae*, which severely damages plants and impairs crop productivity. The bacterium's control method is based on cultural practices preventing the entry of the pathogen in the area and its eradication. Such products as copper oxychloride and mancozeb are weekly applied, although no registered chemicals for control are extant (AMORIM *et al*., 2016).

Difficulties in control, linked to the environment and human health concerns due to agrochemicals have triggered the search for alternative methods for the management of the disease. One approach to alternative agriculture consists in disease control through resistance induction in plants, based on the activation of the plant's defense mechanisms when treated with an inducing agent. The method has been widely studied in the control of phytopathogens (ASHALI *et al*., 2018; KRZYZANIAK *et al*., 2018, LORENZETTI *et al*., 2018).

Mushrooms, such as *Agaricus blazei* and *Lentinula edodes*, are known for their medicinal and nutritional properties. However, studies on biological control and on the induction of plants´ resistance also demonstrate their potential for controlling phytopathogenic microorganisms (SILVA; PASCHOLATI; BEDENDO, 2008; COQUEIRO *et al*., 2011; ARRUDA *et al*., 2012; MENEGASSI, 2017).

Since activation of resistance mechanisms in plants has been studied to control plant diseases, the current paper evaluates *in vitro* effect of *L. edodes* and *A. blazei* extracts on *X. axonopodis* pv. *passiflorae* and resistance induction in passion fruit plants.

2 MATERIAL AND METHODS

The phytobacterium *X. axonopodis* pv. *passiflorae*, retrieved from the Collection of Phytobacterial Cultures - IBSBF of the Plant Bacteriology Laboratory of the Experimental Center of the Biological Institute - Campinas SP Brazil, was used in current research. Bacteria were kept in nutrient agar (NA) medium, in the dark, at 28ºC, for 48h, before inoculations.

Isolates *L. edodes* (LE-95/01) and *A. blazei* (ABL-30) were obtained as dry basidiocarp powder from mushrooms, produced in eucalyptus logs and by axenic cultivation, respectively.

Crude extracts (CEs) were prepared by hydrating the basidiocarp dry powder with distilled water (1:14; p: v) for 24h, at 4°C. Then, the solution was then filtered on filter paper.

2.1 *IN VITRO* EFFECT OF MUSHROOMS ON *X. AXONOPODIS* PV. *PASSIFLORAE*

In vitro tests comprised filtering of aqueous extracts through Milipore® membrane (pore diameter = 0.2 μm) under aseptic conditions. Test tubes with sterile distilled water received aqueous extracts of LE-

95/01 and ABL-30 for final concentrations 10%, 20%, 30% and 40% (v/v). Control treatment consisted of tubes with water only. Subsequently, 1mL of bacterial suspension was added to each tube at a concentration of 10^8 UFC.mL^{-1}. The tubes containing extracts with bacterial suspension were kept in the dark, at 28±2ºC, for 24 h.

Further, 300 μL aliquots from each tube were pipetted onto Petri dishes containing NA medium and then spread over the whole surface with a Drigalsky handle. The plates were kept in the dark at 28±2ºC, for 48h. To evaluate the results, 10 mL of distilled water were added to each plate and the bacterial suspension was measured in a spectrophotometer at 550 nm, obtaining the value of the absorbance unit (AU).

The experiment was conducted in a completely randomized design with four replications, each being a sampling unit. Results underwent analysis of variance by statistical program SISVAR (FERREIRA, 2000) and means were compared by Scott Knott test at 5% probability.

2.2 RESISTANCE INDUCTION IN PASSION FRUIT PLANTS BY AQUEOUS EXTRACTS OF MUSHROOMS

Passion fruit cultivars IAC-275 and Epagri Oval Grande were sown on two substrates: substrate 1 - ravine soil (RB); substrate 2 - 40% ravine soil + 40% organic compost + 20% roughage (RSC). Substrates were corrected with 1.0 kg limestone/m^3 of the substrate and 0.5 kg Yorim/m^3 of the substrate. The pots were kept under a shade screen and the seedlings were watered daily.

Treatments comprised control (without treatment); biofertilizer Agromos® 1% (positive control); aqueous extract of mushroom *A. blazei* 20%; aqueous extract of mushroom *A. blazei* 40%; aqueous extract of mushroom *L. edodes* 20% and aqueous extract of mushroom *L. edodes* 40%. The application of treatments started when seedlings had between 4 and 6 leaves. Pulverization was performed weekly all over the plant to the point of dripping, for four weeks.

Enzyme activity was assessed by collecting leaves seven days after the last spray. Leaves were kept frozen at -20ºC.

The activity of guaiacol peroxidase and protein contents was quantified when samples of plant tissue were homogenized in 2.0 mL of 0.1 M phosphate buffer (pH 6.0) (extraction buffer) and centrifuged at 10,000 rpm for 10 minutes. Supernatants were used as enzymatic extract. Guaiacol peroxidase activity was determined at 30ºC by direct spectrophotometry by measuring the conversion of guaiacol into tetraguaiacol at 470 nm (RONCATTO; PASCHOLATI, 1998). Specific activity was expressed in absorbance variation at 470 nm/min/mg protein. The amount of total proteins contained in the extract was determined following the Bradford method (1976) and expressed in terms of equivalent μg bovine serum albumin (BSA) in 0.8 mL sample (μg protein/0.8mL), using the standard curve of BSA concentrations ranging between 0 and 20 μg/0.8ml.

So that the activity β-1.3 glucanase and chitinase would be quantified, tissue samples from the plants were macerated in liquid nitrogen, homogenized in 4.0 mL of 100 mM sodium acetate buffer (pH 5.0) and centrifuged at 10,000 rpm for 10 min. The supernatant was used to determine the activity of the enzymes.

The activity β-1.3-glucanase was determined by colorimetric quantification of the reducing sugars released by laminarin (VOGELSANG; BARZ, 1993) and the sugars formed were quantified by the Lever method (1972). The result was the difference between the absorbance of tube incubated with laminarin and of the tube incubated without laminarin. The difference in absorbance readings was plotted on a standard glucose curve and the result expressed in μg of glucose/mg of protein.

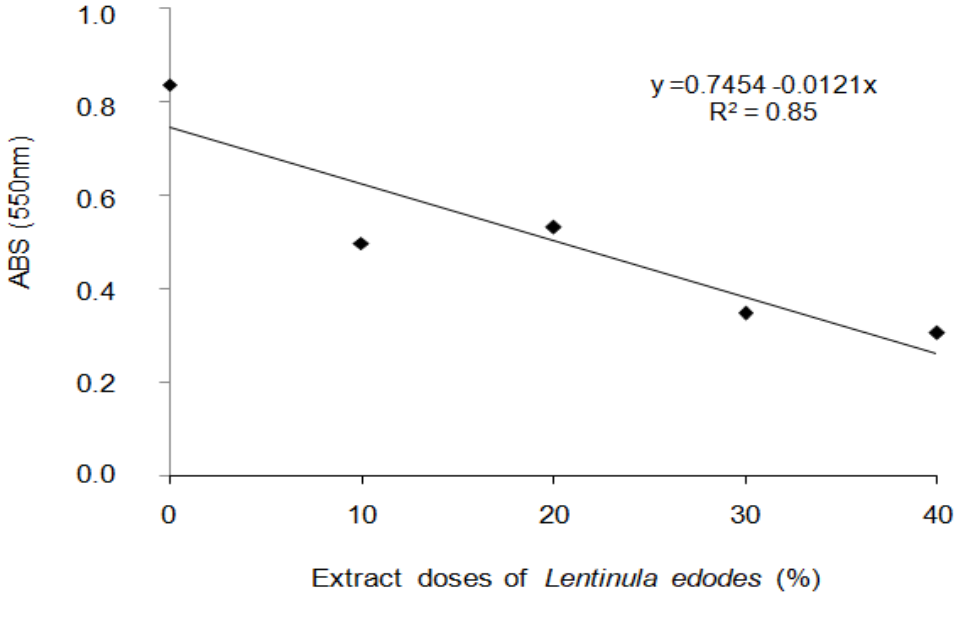
The chitinase activity was evaluated following the Hackman and Goldberg method (1964) by indirect spectrophotometric quantification of the Violet Remazol Chitin Azure (SIGMA). Results were expressed in chitinase units where a chitinase unit corresponded to a variation of 0.001 in the absorbance rate per μg of total protein.

The experiment was carried out in a completely randomized factorial design, 2 (passion fruit cultivars) x 2 (substrates) x 6 (treatments), with 10 replicates. Results underwent analysis of variance by SISVAR (FERREIRA, 2000) and means were compared by Scott Knott test at 5% probability.

3 RESULTS AND DISCUSSION

3.1 *IN VITRO* EFFECT OF MUSHROOMS ON *X. AXONOPODIS* PV. *PASSIFLORAE*

Regression analysis ($p = 0.05$) of absorbance demonstrated that the aqueous extract of *L. edodes* had a linear response, inhibiting bacterial development. Inhibition was dose-dependent since extract concentration was greater (Fig. 1a). Regarding the effect of the aqueous extract of *A. blazei* (Fig.1b), the highest absorbance rate occurred at a concentration of 20% (0.919 UA) and subsequently decreased. Or rather, there was a stimulating effect for the development of the bacteria up to a concentration of 20%, followed by inhibition of bacterial development.



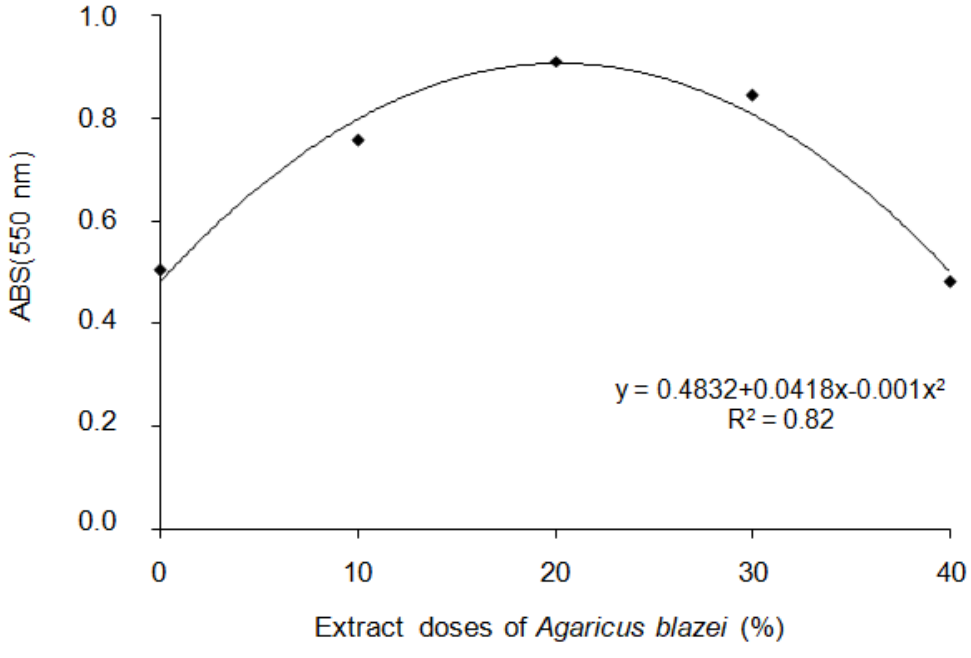


Figure 1. *In vitro* development of *Xanthomonas axonopodis* pv. *passiflorae* (UA) at different doses of (A) *Lentinula edodes* and (B) *Agaricus blazei* extract

One of the main studies to verify the antimicrobial activity of *L. edodes* was carried out by Pacumbaba; Beyl; Pacumbaba (1999) who proved the inhibitory activity of the leachate from *L. edodes* mycelium against several bacteria, such as *Pseudomonas syringae* pv. *glycinea*, *P. syringae* pv. *tabaci*, *X. campestris* pv. *glycine*, *X. campestris* pv. *campestris*, *Erwinia amylovora*, *Ralstonia solanacearum*, *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*, *Bacillus cereus*, *Escherichia coli*, *Listeria monocytogens*, *Salmonella typhimurium* and *Staphylococcus aureus*, corroborating results in the current study, even though the authors did not directly focus research on *X. axonopodis* pv. *passiflorae.*

Piccinin, Di Piero and Pascholati (2010) found that extracts of basidiocarps and *L. edodes* stipe at 2% concentration, incorporated into the culture medium, reduced the multiplication of *X. axonopodis* pv. *passiflorae*. Kwak *et al*. (2015) have investigated *in vitro* growth inhibition of *R. solanacearum* with filtered *L. edodes*, whilst Sasaki *et al*. (2001) detected an inhibitory effect on the fungi *Helminthosporium* sp., *H. euphorbia* and *Phomopsis soyae* by incorporating the aqueous extract of *L. edodes* at concentrations 10%, 20% and 30% in the BDA medium.

By chromatography, Kwak *et al*. (2016) observed the presence of nine organic acids in the culture filtrate of *L. edodes.* Oxalic acid was the main component, exhibiting antibacterial activity against nine different phytopathogenic bacteria, among them *X. axonopodis* v. *glycines* and *X. axonopodis* pv. *citri.* The main components of *Agaricus blazei* are polysaccharides, especially -glucans. These are considered antioxidant agents that protect against oxidative damage and increase the immunity (WEI *et al*., 2019) and can also be a powerful antimicrobial compound.

Piccinin, Di Piero and Pascholati (2010) found that the two aqueous extracts of basidiocarp, as the aqueous extract of pileus stipe of *L. edodes*, showed an inhibitory effect on the sporulation of *Exserohilum turcicum* and *Colletotrichum sublineolum* in concentrations higher than 1%. Oliveira *et al*. (2019) found that aqueous extract of *L. edodes* was effective in controlling anthracnose in common bean at doses of 1% and 5%.

Fiori-Tutida *et al*. (2007) tested *in vitro* several concentrations of *L. edodes* and *A. blazei* extract (0.1%, 1.0%, 10% and 40%) against *Puccinia recondita* f. sp. *tritici* and *Bipolaris sorokiniana* and observed

that mushroom extracts significantly inhibited the spore germination of *P. recondita* f. sp. *tritici.* However, the extracts from the two mushrooms had no significant effect on mycelial growth and on spore germination of *B. sorokiniana.*

Chen and Huang (2011) reported total inhibition of pathogens *Alternaria brassicicola*, *Colletotrichum higginsianum*, *Phytophthora capsici, Pythium aphanidermatum, Rhizoctonia solani, Pectobacterium carotovorum* subsp. *carotovorum* by the *L. edodes* filtrate.

However, divergent results may occur since several mushroom isolates may have a higher or lower concentration of inhibitory substances or differ in the composition and activity of these substances. For example, Silva (2007) performed *in vitro* tests with aqueous extracts of *L. edodes* and *A. blazei* at concentrations 5%, 10%, 15% and 20%, against *Clavibacter michiganensis* subsp. *solanacearum* and founded that none of the extracts inhibited bacterial growth. Extracts stimulated growth in some treatments, and revealed that, unlike a possible antibiotic effect, there was probably an improvement in the nutritional condition of the bacteria development medium.

Using extracts of *L. edodes* and *A. blazei* isolates for *in vitro* testing with *X. vesicatoria*, Di Piero and Pascholati (2004) found that *L. edodes* extracts did not have an inhibitory effect on bacteria and that *A. blazei* extracts even stimulated their growth.

3.2 RESISTANCE INDUCTION IN PASSION FRUIT PLANTS BY AQUEOUS EXTRACTS OF MUSHROOMS

Results of guaiacol peroxidase on cultivar IAC-275 showed that the treatments involving the mushroom *A. blazei* 20% and 40% were superior to control when cultivation was carried out in ravine soil, with a three-fold increment. Treatment with *A. blazei* 20% was superior to control when the cultivation was performed in ravine soil with organic compost (Table 1).

In the case of Epagri Oval Grande cultivar cultivated in ravine soil, the treatments with *A. blazei* 20% and *L. edodes* 40% were superior to control. For ravine soil plus organic compost, the treatments with *A. blazei* 20% and 40%, *L. edodes* 40% and Agro-Mos® (positive control) were statistically superior to control. Agro-Mos® was superior to control only with ravine soil + organic compost and more than two-fold greater than the rate for ravine soil (Table 1).

Table 1. Specific guaiacol peroxidase activity (470nm / min / mg protein) in passion fruit leaves of IAC–275 and Epagri Oval Grande cultivars, treated with mushroom extracts and grown in ravine soil (RS) and ravine soil + organic compost (RSC)

Cultivars		Control	Agro-Mos®	*Agaricus blazei*		*Lentinula edodes*	
				20%	40%	20%	40%
IAC–275	**RS**	0.4059 b	0.6087 b	1.1510 a	1.1278 a	0.5281 b	0.5059 b
	RSC	0.5467 b	0.7433 b	1.6716 a	0.7234 b	0.8775 b	0.8094 b
Epagri Oval Grande	**RS**	0.5982 b	0.3728 b	0.8490 a	0.4609 b	0.5557 b	0.8819 a
	RSC	0.5213 b	0.7960 a	0.6764 a	0.7457 a	0.5365 b	0.8075 a

Means on the lines followed by the same letter do not differ by Scott-Knott test at 5% probability.

The specific activity of peroxidase on the cultivar Epagri Oval Grande showed a higher rate in plants cultivated in a ravine soil with organic compost. Rates obtained by untreated plants (controls) were statistically

the same and indicated that the substrate may interfere with the enzyme's activity when performed with the treatments with mushrooms. When the performance of the two varieties is compared, guaiacol peroxidase's activity increments were higher in the IAC-275 cultivar than in the Epagri Oval Grande cultivar.

Oliveira *et al*. (2019) observed that aqueous extract of *L. edodes* was effective in activating the peroxidase, catalase and polyphenoloxidase enzymes, with a 20% dose standing out. Garcia *et al.* (2018) demonstrated that the 10% dose of *Agaricus brasiliensis* aqueous mycelial suspension (mushroom of the same genus) reduced catalase activity and induced peroxidase activity. However, it was not enough to reduce the severity of Isabel Precoce grape downy mildew, even acting on enzyme activity.

Although not all the biological functions of peroxidase are fully understood, it is known that they play an important role in the biosynthesis and lignification of the secondary cell wall related to pathogen resistance. Specific peroxidases, such as those from guaiacol, catalyze the oxidation of phenolic alcohols to lignin and reduce hydrogen peroxide to water (RESENDE *et al*., 2007).

The activity of enzyme β-1.3-glucanase was differentiated according to cultivar and soil type. In fact, the activity of β-1.3-glucanase (Table 2) for cultivar IAC-275 cultivated in ravine soil showed a significant increase in relation to control for treatments with *L. edodes* 20% and 40% and control. Nevertheless, the rate obtained by treatment with *A. blazei* 20% was significantly lower. In ravine soil plus organic compost, the treatments with *A. blazei* 40% and *L. edodes* 20% and 40% presented higher rates than those of control. Results revealed that mushroom *L. edodes*, at both concentrations, provided a higher activity of β-1.3-glucanase in the two types of soils tested.

Table 2. Specific activity of β-1,3-glucanase (μg glucose / mg protein) in leaves of passion fruit plants, cultivars IAC–275 and Epagri Oval Grande, treated with mushroom extracts and cultivated in ravine soil (RS) and ravine soil + organic compost (RSC)

Cultivars		Control	Agro-Mos®	*Agaricus blazei*		*Lentinula edodes*	
				20%	40%	20%	40%
IAC–275	RS	0.560 b	1.031 a	0.293 c	0.524 b	0.871 a	0.887 a
	RSC	0.537 b	0.533 b	0.432 b	0.710 a	0.716 a	0.881 a
Epagri Oval Grande	RS	0.717 a	0.849 a	0.830 a	0.852 a	0.377 b	0.489 b
	RSC	0.928 a	0.549 b	0.663 b	0.398 b	0.541 b	0.547 b

Means on the lines followed by the same letter do not differ by Scott-Knott test at 5% probability.

The activity of β-1.3-glucanase in the Epagri Oval Grande cultivar did not show a significant increase in the different inducing treatments and in the two soil types when compared to those of control. Moreover, in ravine soil, treatments with *L. edodes* 20% and 40% also provided a significant decrease in rates when compared to control. In ravine soil plus organic compost, all treatments showed rates significantly lower than control.

Melo *et al.* (2020) verified an increase in the β-1,3-glucanase, peroxidase and phenylalanine ammonia lyase (PAL) activity, in tomato treated with β-glucan extracted from *L. edodes.* This demonstrates that this compound, also present in large quantities in *A. blazei* (WEI *et al.*, 2019), may be associated with the pathogenesis-related proteins induction.

Studies by Guimarães *et al.* (2010) demonstrated that the activity of enzyme β-1,3-glucanase was low in induced sugarcane plants and had no significant increase on different days after induction when compared with control. This response corroborates the present study. Similarly, in their studies on the pathosystem of bean/*X. axonopodis* pv *phaseoli*, Kuhn and Pascholati (2010) observed that acibenzolar-S-methyl increased the activity of peroxidase, chitinase and β-1,3-glucanase, while *Bacillus cereus* only increased peroxidase.

Different results founded by Di Piero and Pascholati (2004b) who detected that the aqueous extract of an isolate of *A. blazei* significantly reduced the severity of the bacterial spot on the tomato, in greenhouse, when the extract was applied five days before inoculation. They also reported an increase in the activity of -1,3-glucanase, suggesting induction of resistance in tomatoes against *X. vesicatoria*

In the case of chitinase activity, treatments with *A. blazei* 20% and 40%, IAC-275 cultivar cultivated in ravine soil, promoted a significant rate increase when compared to control. Other treatments of the cultivar did not differ significantly from the control. No treatment differed statistically from control when cultivar Epagri Oval Grande is taken into account (Table 3). The above may indicate that the enzyme was incapable of indicating resistance when aqueous extracts of *L. edodes* and *A. blazei* mushrooms were used as resistance inducers. There was practically no accumulation of the enzyme in plants treated with mushrooms and with resistance inducer Agro-Mos®.

Table 3. Specific activity of chitinase in passion fruit leaves cultivar IAC–275 and Epagri Oval Grande, treated with mushroom extracts and cultivated in ravine soil (RS) and ravine soil + organic compost (RSC)

Cultivars		Control	Agro-Mos®	*Agaricus blazei*		*Lentinula edodes*	
				20%	40%	20%	40%
IAC–275	RS	1.105 b	1.338 b	1.811 a	2.083 a	1.458 b	1.561 b
	RSC	1.123 b	1.207 b	1.239 b	1.521 b	1.277 b	1.393 b
Epagri Oval Grande	RS	0.971 a	1.639 a	1.343 a	1.357 a	1.347 a	1.420 a
	RSC	1.019 a	1.176 a	0.988 a	1.337 a	1.284 a	1.195 a

* Rates were calculated by summing data of the first collection (7 days after the first treatment) and the second collection (7 days after the last treatment). Means on the lines followed by the same letter do not differ by Scott-Knott test at 5% probability.

The enzymes chitinase and β-1,3-glucanase have a direct action against the pathogen, degrading the cell wall and impairing the establishment of stable parasitic relationships and colonization (KUHN; PASCHOLATI, 2010). However, Di Piero (2003) reports that the protection of tomatoes against *X. vesicatoria* induced by acibenzolar-S methyl and *A. blazei* remains uncertain, since the pathogen does not have chitin or β-1,3-glucan in its cell wall. According to the author, these enzymes may not have a direct effect on the bacteria, but may contribute to the establishment of an unfavorable environment for the pathogen in the intercellular spaces where colonization occurs.

The activity of studied enzymes has also been observed in passion fruit plants, even in non-induced (control) plants. It is important to remember that some pathogenesis-related proteins may be present in the plant's constitution, albeit at low concentrations, with a significant increase as a result of inducing their synthesis by plant or pathogen-eliciting substances (XUE; CHAREST; JABAJI-HARE, 1998).

The current study showed no inoculation with the pathogen. In an attempt to obtain the same conditions as the farmer would have on the field, only treatments with inducing agents were performed to

verify the occurrence of the enzymatic activity without the inoculation of the pathogen. This fact may have provided a lower amount of enzymatic activity when compared to tests in which association with the pathogen occurs. Several authors obtained positive results on enzymatic activity when, after the inducement of plants, they inoculated the pathogen (ITAKO *et al*., 2012; ARAUJO; STADNICK, 2013; MELLO *et al*., 2017).

Varying response to enzymatic activity depends on the cultivar. Guerra *et al*. (2013) evaluated that the activity of peroxidase, chitinase and β-1,3-glucanase enzymes varied in relation to cultivars BRS Araçá and FM 993 submitted to silicon treatments in the resistance of cotton to *Ramularia areola.*

Although in the current study the enzymatic activity of guaiacol peroxidase and β-1,3-glucanase varied according to the cultivar used, the type of soil and the concentration of the extracts used, the aqueous extracts mushrooms *L. edodes* and *A. blazei* showed that they may induce the enzymes´ activities. These results corroborate those obtained by Di Piero (2003), who investigated the potential of *L. edodes* and *A. blazei* in the control of cucumber, passion fruit and tomato diseases and detected that these mushrooms have defense-inducing substances and may help in disease control.

4 FINAL CONSIDERATIONS

The *X. axonopodis* pv. *passiflorae* inhibition caused by *L. edodes* was dose-dependent, whereas of *A. blazei* the highest absorbance rate occurred at a concentration of 20%.

Results of guaiacol peroxidase showed that the treatment involving the mushroom *A. blazei* 20% was superior to control in all cases. In the case of *L. edodes,* only 40%, in the cultivar Epagri Oval Grande, was superior to control. Results of β-1,3-glucanase showed that the treatment involving the mushroom *A. blazei* 40% was superior to control, in the cultivar IAC-275 planted in ravine soil+organic compost. In the case of *L. edodes* 20% and 40% were superior to control, only in the cultivar IAC-275. Results of chitinase showed that the treatments involving the mushroom *A. blazei* 20 and 40% were superior to control, in the cultivar IAC-275 planted in ravine soil. In the case of *L. edode,* there was no activation.

The aqueous extracts of *L. edodes* and *A. blazei* showed an inhibitory effect, *in vitro*, on *X. axonopodis* pv. *passiflorae* and increased guaiacol peroxidase and β-1,3-glucanase activity according to cultivar and soil type. Thus, the aqueous extracts of the mushrooms *L. edodes* and *A. blazei* are promising for resistance induction to control the bacterial spot of passion fruit.

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