

Lentinula edodes extract in the control and induction of resistance to common bean pathogens

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ABSTRACT: Common bean (*Phaseolus vulgaris* L.) is among the main crops of national agriculture and can be affected by several diseases. Aiming to develop alternative control methods to chemical control, it was verified the potential of the aqueous extract (AE) from *Lentinula edodes* in controlling common bean diseases and inducing resistance by the activation of defense enzymes such as peroxidase, catalase and polyphenoloxidase, in addition to evaluating production components. The field experiment consisted of 0, 5%, 10% or 20% acibenzolar-S-methyl (ASM) and fungicide pyraclostrobin treatments as standard treatments. AE was effective in controlling anthracnose at 1% and 5% doses. The activity of all evaluated enzymes was higher at 12 and 24 hours after application of the treatments, with a concentration of 20% standing out. AE at this same dose increased the number of beans per pod and also yield. The aqueous extract of *Lentinula edodes* at a concentration of 20% is effective in inducing resistance, increasing enzyme levels and increasing yield.

Key words: alternative management; *Colletotrichum lindemuthianum*; *Phaseolus vulgaris*

Extrato de *Lentinula edodes* no controle e na indução de resistência a patógenos do feijoeiro

RESUMO: O feijoeiro (*Phaseolus vulgaris* L.) está entre as principais culturas da agricultura nacional e pode ser afetada por diversas doenças. Com o objetivo de desenvolver métodos de controle alternativos ao químico, verificou-se o potencial do extrato aquoso (EA) de *Lentinula edodes* no controle de doenças do feijoeiro e na indução de resistência pela ativação de enzimas de defesa, como peroxidase, catalase e polifenoloxidase, além de avaliar componentes de produção. O experimento em campo foi constituído pelos tratamentos com EA nas concentrações de 0, 5%, 10% ou 20%, acibenzolar-S-metil (ASM) e fungicida piraclostrobina como tratamentos padrões. O EA foi efetivo no controle da antracnose nas doses de 1% e 5%. A atividade de todas as enzimas avaliadas foi mais elevada as 12 e 24 horas após aplicação dos tratamentos, destacando-se a concentração de 20%. O EA nesta mesma dose aumentou o número de grãos por vagem e a produtividade. O extrato aquoso de *L. edodes* na concentração de 20% é efetivo na indução de resistência, aumentando os níveis enzimáticos e incrementando produtividade.

Palavras-chave: manejo alternativo; *Colletotrichum lindemuthianum*; *Phaseolus vulgaris*

Introduction

The common bean plant (*Phaseolus vulgaris*) is widely cultivated in southern Brazil, and it is one of the most consumed grains in daily human food, mainly due to its high protein and iron content (Pereira et al., 2015). One of the biggest problems that compromise crop yield is the disease susceptibility. Anthracnose (*Colletotrichum lindemuthianum*), angular leaf spot (*Pseudocercospora griseola*) and common bacterial blight (*Xanthomonas axonopodis* pv. *phaseoli*) are the most important diseases of the crop (Ribeiro et al., 2016; Kijana et al., 2017; Ribeiro et al., 2017).

Several control methods can be used in the management of these diseases, however, the use of pesticides is still the most common. This form of disease control can be very effective in the short term, but can also cause environmental damage and/or pathogen resistance to its active ingredient over time. Thus, an alternative method to the use of pesticides is necessary, with the resistance induction being a way to control phytopathogens by the application of an eliciting agent, which triggers different events in the plant in order to increase the action of defense mechanisms (Stangarlin et al., 2011).

Among the eliciting agents, the extracts from basidiomycetes have been distinguished, as they can act directly on the development of pathogens and also have the potential to increase plant resistance levels by different mechanisms (Garcia et al., 2018). Silva et al. (2007; 2008), when studying the application of aqueous extracts from *Lentinula edodes* and *Agaricus blazei* on eggplant and tomato plants in the control of *Ralstonia solanacearum*, observed an increase in chitinase and peroxidase activity. These enzymes are PR-proteins capable of inducing resistance to plants, having direct action on the pathogen or increasing the barrier that it must penetrate. Viecelli et al. (2009; 2010) also observed an increase in peroxidase and polyphenoloxidase activity after application of *Pycnoporus sanguineus* extracts in common bean, decreasing angular leaf spot severity.

However, besides these facts, it is interesting to observe the effect of basidiomycete extracts on yield. Therefore, this study had as objective to investigate the potential of *L. edodes* aqueous extract in controlling common bean diseases and in inducing resistance through the activation of the peroxidase, catalase and polyphenoloxidase enzymes, in addition to evaluating the production components.

Materials and Methods

Experiment locations

The experiments were conducted at the Plant Pathology Laboratory from the Department of Agronomy, and in the field at the State University of the Central West, CEDETEG campus, under the geographic coordinates of 25°23'36"S and 51°27'19"O, having approximately 1,120 m of altitude. The climate is classified as subtropical humid subtropical (Cfb) according to Köppen, without a dry season, with cool summers and moderate winter. The soil of the experimental area is an Oxisol ("Latossolo Bruno", according to Embrapa).

Treatments and extracts preparation

The dried basidiocarp of the *L. edodes* mushroom was commercially acquired and then ground in a 1 mm sieve knife-mill. The resulting dried powder was hydrated at the 1 g 14 mL⁻¹ proportion of distilled water for 24 h at 4 °C. After hydration, the crude aqueous extract was filtered on filter paper. Subsequently, it was diluted with distilled water in order to obtain the final concentrations. As for the treatments, the following were used: 1) negative control: water, 2) positive controls: acibenzolar-S-methyl (ASM, 200 mg L⁻¹ of the commercial product) and piraclostrobin fungicide of the strobirulin class (0.3 L ha⁻¹ of the commercial product), and 3) the doses of 1%, 5%, 10% and 20% of the aqueous extract (AE) from *L. edodes*.

Experiment in vivo

The field experiment consisted of three randomized blocks, each block consisting of seven plots, and with each plot within a block representing one treatment, totaling seven treatments. Each plot had four rows of 4 m length, spaced 0.5 m apart themselves. The two central lines, discounting 0.5 m from the anterior and posterior borders, were considered as useful area for all evaluations. The IPR Touiuiu cultivar was used and 480 kg ha⁻¹ of NPK (04-14-08) were applied into the sowing furrow, with other cultural treatments, such as manual weeding, performed whenever necessary.

Treatments were applied to the entire plot with a CO₂ pressurized backpack sprayer equipped with a 2 m bar and 4 Jacto® J5-2 empty cone nozzles (J5 disc, 15 mm of outside diameter), with travel speed of 3.6 km h⁻¹ and syrup volume of 200 L ha⁻¹. Applications were carried out at the vegetative stage (V4 – third trifoliolate leaf is fully open and flat, with the development of the first secondary branches observed) and at the reproductive stage (R6 – stage where 50% of the flowers are open in the plant).

Effect of *L. edodes* AE in controlling anthracnose, bacterial blight and angular leaf spot in field

Disease severity was evaluated at 54, 57, 60, 63, 68 days after sowing (DAS) and at 2, 5, 8, 11, 16 days after the second application (DAA) of the treatments, after natural occurrence of the diseases symptoms (no artificial inoculation) through diagrammatic scales elaborated by Godoy et al. (1997), Dalla Pria et al. (2003) and Lima et al. (2013), obtaining five evaluations from the lower middle portion of the plants. Having the disease severity evaluations, its progress curve was constructed and the area under the disease progress curve (AUDPC) was determined (Campbell & Madden, 1990). Afterwards, the treatments efficiency when compared to the control was determined by the Abbott equation (1925).

Effect of the *L. edodes* AE in inducing resistance

In order to perform the biochemical analyzes, the samples were collected in the second application, 52 days after sowing (DAS). Leaf discs with 12 mm in diameter (five discs plot⁻¹) were collected at 12 hours before application (HBA), 3, 6, 12 and

24 hours after application (HAA). During the procedure, each collected sample was immediately placed in foil envelopes and frozen with liquid nitrogen. Samples were collected from the central leaflet of the upper trifolium.

For obtaining the protein extracts, leaf samples were mechanically homogenized in 2 mL of 0.01M potassium phosphate buffer (pH 7.0) (extraction buffer) in a porcelain mortar. This solution was centrifuged at 13,500 g for 30 min at 4 °C, and the obtained supernatant was considered as enzymatic extract for subsequent determination of the activity of peroxidase, catalase, polyphenoloxidase, and protein content.

Specific activity of guaiacol peroxidases (POX, EC 1.11.1.7) was determined at 30 °C in a spectrophotometer at 470 nm for 2 min. The solution mixture consisted of 2.9 mL solution containing 250 µL of guaiacol and 306 µL of hydrogen peroxide (H₂O₂) in 100 mL of 0.01 M potassium phosphate buffer (pH 6.0) and 100 µL of enzyme preparation (Lusso & Pascholati, 1999). Specific enzyme activity was expressed in units of absorbance per minute per mg of protein (U.A. min⁻¹ mg⁻¹ protein).

Catalase specific activity (CAT, EC 1.11.60) was determined by quantifying the stable complex composed by ammonium molybdate with hydrogen peroxide (Tomanková et al., 2006). The enzyme extract (100 µL) was incubated in a hot water bath at 38 °C along with 0.5 mL of the reaction mixture containing hydrogen peroxide in 0.06 M potassium phosphate buffer (pH 7.4) for 2 min. After this period, 0.5 mL of ammonium molybdate was added to deter the hydrogen peroxide consumption by the enzyme present in the extract. A blank was prepared for each sample by adding ammonium molybdate to the reaction mixture, omitting the incubation period. The yellow molybdate complex and the hydrogen peroxide were measured at 405 nm. The difference between the absorbance of the blank and the incubated sample indicated the amount of hydrogen peroxide used by the enzyme. H₂O₂ concentration was determined by using the extinction coefficient $\epsilon = 0.0655 \text{ mM cm}^{-1}$. Specific enzyme activity was expressed as $\mu\text{mol min}^{-1} \text{ mg}^{-1} \text{ protein}$.

Polyphenoloxidase specific activity (PPO, EC 1.10.3.1) was determined at 30 °C in a spectrophotometer at 420 nm for 2 min. The solution mixture consisted of 0.9 mL of solution containing 1.001 g of catechol in 50 mL of 0.01 M potassium phosphate buffer (pH 6.8) and 100 µL of enzyme preparation (Duangmal & Aparenten, 1999). Specific enzyme activity was expressed in units of absorbance per minute per mg of protein (U.A. min⁻¹ mg⁻¹ protein).

Total protein content was evaluated by the Bradford method (1976). Thus, the Bradford reagent preparation was used with the addition of ethanol, phosphoric acid and G-250 Coomassie Bright Blue coloring agent. The interaction between high molecular weight proteins and the coloring agent causes the equilibrium displacement of the coloring agent from anionic to cationic form, which allows the absorption of the 595 nm wavelength (Zaia et al., 1998). Protein concentration, expressed as equivalent µg from albumin of bovine serum (ABS) in one mL of sample (µg protein mL⁻¹), was determined by using the standard ABS concentration curve.

Production components

At harvest, grain yield and yield components (number of pods per plant, number of grains per pod, and weight of thousand grains) were evaluated. Grains from the manual threshing of all pods supplied the seed yield thought weighting (corrected to 13% humidity and transformed into kg ha⁻¹). All production components were determined in the usable area of 8 m² of the plot.

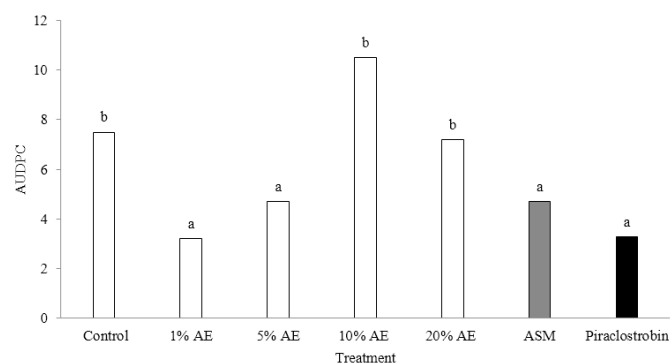
Statistical analysis

Analysis of variance was performed by employing the statistical program SISVAR (Ferreira, 2011) and the comparison of means between treatments was performed by the Scott-Knott test at 5% probability.

Results and Discussion

Effect of *L. edodes* AE on the control of anthracnose, bacterial blight and angular leaf spot in the field

In relation to the anthracnose control, the statistical difference was significant, with the lowest doses being statistically equal to the fungicide and to the acibenzolar-S-methyl (ASM), with the concentration of 1% of AE having decreased by 58% and of 5% in 37% the AUDPC of the disease when compared to the control (Figure 1 and Table 1). There was no statistical difference for bacterial blight control and angular leaf spot (data not shown). It is noteworthy that



** Significant at 5% probability by F test. Means followed by the same letter do not differ from each other by the Scott-Knott test ($p \geq 0.05$).

Figure 1. Area below the disease progress curve (AUDPC) of anthracnose in common bean under field conditions, treated with aqueous extract (AE) of *Lentinula edodes*, acibenzolar-S-methyl (ASM) and piraclostrobin fungicide.

Table 1. Area below the anthracnose disease progress curve (AUDPC) in common bean under field conditions, treated with aqueous extract (AE) of *Lentinula edodes*, acibenzolar-S-methyl (ASM) and piraclostrobin fungicide.

Treatments	Efficiency (%)
1% AE	58
5% AE	37
10% AE	140
20% AE	5
ASM	37
Piraclostrobin	56

for the development of these diseases, favorable climatic conditions are required, which may influence the absence of disease or low AUDPC as observed for anthracnose. (Siqueira et al., 2019).

This observed control effect with *L. edodes* AE applications may be associated with the composition of this mushroom, rich in bioactive metabolites such as polysaccharides, which trigger antimicrobial effects (Sharma et al., 2016). Since this mushroom can also present mycelial growth on pathogens such as *Verticillium* sp. and *Phythium* sp. (Owaid, 2017), a fact that may have occurred in the present study that contributed to the reduction of anthracnose, by the biological control action by mycoparasitism. However, these processes depend on the sensitivity of the pathogen to the bioactive compounds released by *L. edodes* (Kang et al., 2017).

Similar results were found by Viecelli et al. (2010), that when applying aqueous extract of *P. sanguineus* mycelium at concentrations of 10% and 20% in the field bean did not obtain control of angular spot in the lower portion of the plants. Piccinin et al. (2010) obtained control over the pathogens *Exserohilum turcicum* and *Colletotrichum sublineolum* using the concentration of 2% of aqueous extract from *L. edodes* basidiocarp, reducing the severity of sorghum leaf diseases. Kaur et al. (2016) point out that *L. edodes* mycelium filtrate inhibits 100% of the *Xanthomonas campestris*, causal agent of tomato bacterial spot. These results prove the biological action of this extract on phytopathogens.

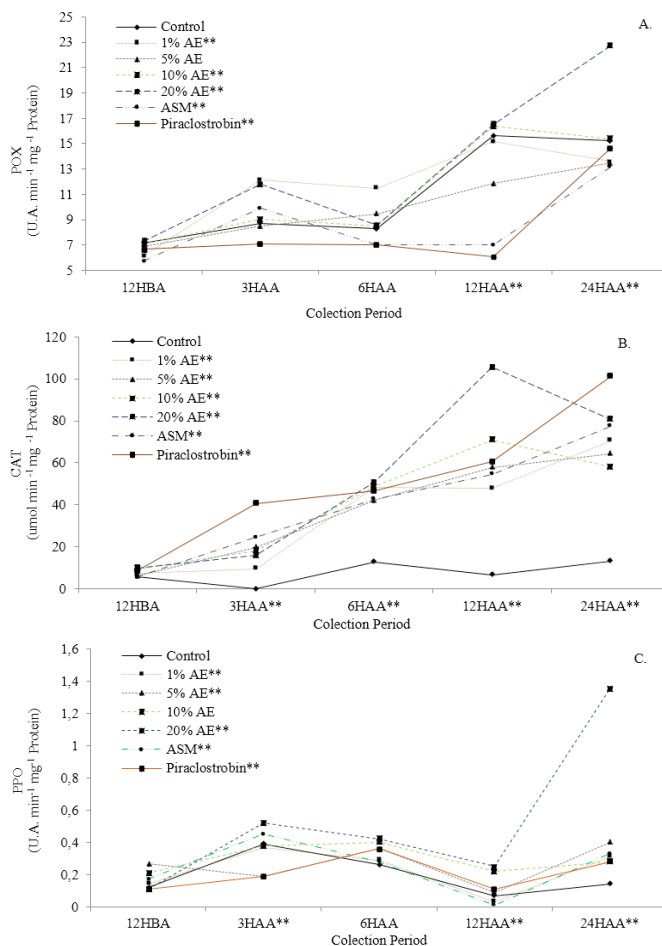
Effect of the *L. edodes* AE in inducing resistance

From the values obtained in the determination of peroxidase (POX), catalase (CAT) and polyphenoloxidase (PPO), the applied treatments presented significant statistical differences. For the treatment with 20% AE, the activity response of all evaluated enzymes exceeded the maximum value obtained through the other treatments, peaking at 12 and 24 hours after application (HAA) (Figure 1).

For the peroxidase enzyme, the mean value reached by the treatments was around 15 U.A.⁻¹min⁻¹ mg protein, while with the 20% treatment, 24 HAA, there was a significant increase in enzymatic activity, with a value above 22 U.A.⁻¹min⁻¹ mg protein, differing statistically from other treatments (Figure 1A). In the catalase activity, the enzymatic peak reached through this treatment occurred at 12 HAA, which was statistically higher, with a value of approximately 105 umol⁻¹ min⁻¹ mg protein, surpassing the mean of the other treatments (80 umol⁻¹ min⁻¹ mg protein) (Figure 1B). The 20% concentration of the extract resulted in an increase in polyphenoloxidase activity at 12 and 24 HAA, differing statistically from the other treatments, and passing the mean values of 0.4 U.A.⁻¹min⁻¹ mg protein of these by approximately 237%. (Figure 1C).

This oscillation in enzyme activity after the application of basidiomycete extracts as elicitor is common and found in several studies (Silva et al., 2007; Silva et al., 2008; Viecelli et al., 2009; Toillier et al., 2010 Viecelli et al., 2010).

Peroxidases are enzymes responsible for the resistance or susceptibility of plants in different pathosystems. When in high activity, as observed in 24 HAA, phenolic compounds that



** Indicates significant statistical difference by Scott-Knott test at 5% probability level. HBA: hours before application and HAA: hours after application treatments.

Figure 2. Specific activity of peroxidase (POX), catalase (CAT) and polyphenoloxidase (PPO) in bean plants treated with aqueous extract (AE) of *Lentinula edodes* at concentrations 0, 1, 5, 10 and 20%, acibenzolarS-methyl (ASM) and pyraclostrobin fungicide.

act directly on the pathogen may reduce oxidation, reducing its development and synthesis of lignin in the host plant. Although *C. lindemuthianum* itself, can induce the activity of these enzymes through mechanical disruption or production of proteolytic enzymes at the time of penetration. However, POX activity in the control was low, making it clear that the treatments applied to these plants induce it (Minibayeva et al., 2015; Andreasson & Laith, 2017).

Increased activity of antioxidant enzymes, such as peroxidase, catalase and polyphenoloxidase, reflects a higher stress condition, which was observed in the study with the application of higher extract concentration. Decreased catalase or peroxidase activity may lead to an increase in the amount of hydrogen peroxide in cells, which may cause cell death, characterized as a hypersensitivity response (Camejo et al., 2016). When the elicitor comes into contact with the plant cell, there is an increase in ionic flux, protein phosphorylation/dephosphorylation, and production of signaling molecules such as jasmonic acid, salicylic acid, ethylene and reactive oxygen species (Kuhn & Panstruga, 2014). Therefore, the

Table 2. Number of grains/pod of bean treated with aqueous extract of *L. edodes*, acibenzolar-S-methyl (ASM) and fungicide.

Treatments	Pods/Plant (no.)	Grains/Pods** (no.)	Weight of thousand grains** (G)	Yield** (kg ha ⁻¹)
Control	10.28 a	5.05 b	255.87 a	2030.6 b
1% AE	10.73 a	5.79 a	233.7 a	2223.7 b
5% AE	12.86 a	4.83 b	229.2 a	2023.8 b
10% AE	12.98 a	6.36 a	210.17 b	2579.9 a
20% AE	12.3 a	6.3 a	210.27 b	2475.9 a
ASM	14.13 a	5.43 a	233.9 a	2747.7 a
Piraclostrobin	11.53 a	5.61 a	243.1 a	2413.3 a
CV (%)	18.57	8.65	5.28	12.87

** Significant at the 5% probability by the F test. Means followed by the same letter do not differ from each other by the Scott-Knott test ($p \geq 0.05$).

high enzymatic levels found after elicitor application reflect increased defense mechanisms for future stress conditions that the plant may face.

Production components

For the production components, extract at concentrations of 1%, 10% and 20% increased the number of grains per pod, being statistically equal to the standard fungicide treatment. However, for mass of one thousand grains, the doses of 10 and 20% two reduced this value. For yield, the 10% and 20% AE did not differ from the two standard treatments with ASM and Piraclostrobin, increasing by 27%, 22%, 35% and 19%, respectively (Table 2).

Reduction of the weight of thousand grains caused by the higher doses of the extract may be related to the activation of the defense enzymes of bean plants. This process demands an energy cost that can reflect this variable in common bean (Kuhn & Pascholati, 2010).

There are few studies relating the use of resistance inducers and plant yield. The use of *L. edodes* applications in pepper activated defense mechanisms in these plants and promoted growth by increasing leaf number, fresh root and shoot weight and height by 40% (Kang et al., 2017).

In the present study, there was an increase in the activity of antioxidant enzymes, which can act as anti-stress in plants. Increased activity of oxidative stress enzymes is observed by the application of growth bioregulators (Zavaleta-Macera et al., 2007), which favor development (Albrecht et al. 2012), increasing yield in the applied crop.

Conclusions

The aqueous extract from *L. edodes* at concentrations of 1% and 5% reduced the anthracnose AUDPC. However, the 20% concentration increased the activity levels of the enzymes POX, CAT and PPO, which compensated the stress caused by the phytopathogen attack, increasing the bean yield.

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