https://doi.org/10.1590/1678-4499.20240276

CROP PROTECTION
Article

Use of fungal filtrates in tomato defense against *Alternaria linariae*, the causal agent of early blight pathogen

Amanda Felchak de Morais[1,*], Karla Siebert Sapelli[1], Kátia Regina Freitas Schwan-Estrada[2], Sergio Miguel Mazaro[3], Cacilda Márcia Duarte Rios Faria[1]

1. Universidade Estadual do Centro-Oeste – Agronomia – Guarapuava (PR), Brasil.
2. Universidade Estadual de Maringá – Agronomia – Maringá (PR), Brasil.
3. Universidade Tecnológica Federal do Paraná – Agronomia – Pato Branco (PR), Brasil.

Received: Jan. 9, 2025 | **Accepted:** Aug. 20, 2025

Section Editor: Fabrício de Ávila Rodrigues

***Corresponding author:** amandafelchak@hotmail.com

How to cite: Morais, A. F., Sapelli, K. S., Schwan-Estrada, K. R. F., Mazaro, S. M. and Faria, C. M. D. R. (2025). Use of fungal filtrates in tomato defense against *Alternaria linariae*, the causal agent of early blight pathogen. Bragantia, 84, e20240276. https://doi.org/10.1590/1678-4499.20240276

ABSTRACT: Tomato (*Solanum lycopersicum* L.) is one of the most economically important crops in the world, with its consumption increasing annually due to its multiple uses. This increases phytosanitary problems considerably, such as the presence of pathogens like *Alternaria linariae*, responsible for the disease known as early blight, one of the most aggressive in this crop, resulting in the excessive consumption of fungicides. In recent years, the use of preparations with non-pathogenic fungi among other chemical products as elicitors has become an important control strategy to induce plant resistance. Thus, the aim of this study was to evaluate the action of fungal biomass filtrates in inducing resistance in tomato plants grown in a greenhouse and inoculated with *A. linariae*. The treatments were: control; inoculated control; biomass filtrates of *Ganoderma lucidum* 20%; *Pleurotus ostreatus* 20%; and *Trichoderma asperellum* 20%. *Ganoderma lucidum* filtrate stood out among all treatments, demonstrating potential for inducing resistance in tomato plants by activating the enzymes superoxide dismutase, peroxidase, and catalase, components of the plant's antioxidant system, avoiding lipid peroxidation. Such induction is related to the association between elicitors and the activation time of these routes. Further research is needed to identify the specific bioactive compounds present in fungal filtrates responsible for resistance induction and to elucidate their modes of action.

Key words: biocontrol, elicitor, *Ganoderma lucidum*, *Pleurotus ostreatus*, *Solanum lycopersicum* L., *Trichoderma asperellum*.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most widely produced and most important horticultural crops and a rich source of vitamins A and C (Boccia et al. 2019). However, tomato diseases such as tomato early blight, caused by the pathogen *Alternaria linariae*, can lead to significant losses in the economic value of the crop due to its destructive potential, damaging leaves with characteristic dark, target-shaped spots that enlarge and cause early abscission with no genetic resistance due to the difficulty of crossing species (Astani et al. 2022, Adhikari et al. 2024, Schmey et al. 2024).

The most common approach to controlling early blight is the frequent use of fungicides, but resistant strains in *Alternaria* spp. populations are selected and may pose a threat to intensive production systems (da Silva Junior et al. 2023). A viable and sustainable alternative is the use of biofungicides composed of fungi to manage tomato early blight, and, in recent years, several fungal elicitors have been identified due to their ability to trigger the activation of plant immunity signaling pathways (Patel et al. 2020, da Silva et al. 2021).

During the interaction between plants and elicitors, immune responses such as the production of reactive oxygen species (ROS) can be observed, which is considered the initial signal of multiple immunological pathways in plants, with the ability

to increase ion flow, nitric oxide (NO) production, increase extracellular pH, stomata closure, cell wall strengthening, and the accumulation of pathogen-related proteins (PRPs), playing an important role in the plant's immune response (Nabi et al. 2021, Pring et al. 2023).

In response to ROS production, plants have developed a strong elimination system, ensuring that the content of these molecules when produced at high speed in tissues is not excessive to the point of leading to lipid peroxidation (Dvořák et al. 2021, Kumar et al. 2021). Thus, the aim of this work was to determine lipid peroxidation, and the activity of antioxidant enzymes related to the induction of resistance in tomato plants treated with fungal biomass filtrates and inoculated with *A. linariae*.

MATERIAL AND METHODS

Experiment location

The experiments were conducted from August 8 to December 16, 2022, in two greenhouses simultaneously (experiments 1 and 2), with optimal conditions: temperatures between 18 and 30°C and a relative humidity between 40 and 90%, approximately, both located on the CEDETEG *campus* of the Universidade Estadual do Centro-Oeste (UNICENTRO), in Guarapuava, Paraná, Brazil, where the evaluations were conducted.

In-vitro evaluations were carried out in the Phytopathology Laboratory of the Department of Agronomy, with geographic coordinates: latitude 25°23'01.7"S and longitude 51°29'18.5"W. The climate of the experimental region is classified as Cfb, humid mesothermal subtropical, according to the Köppen classification (Aparecido et al. 2016).

Isolation of fungi and preparation of fungal biomass filtrates

The fungus *Trichoderma asperellum* isolate was obtained from the collection of the Phytopathology Laboratory from the region where the experiment was conducted. It already has proven efficacy in relation to its ability to promote growth, as well as reduce the severity of diseases in plants (Pittner et al. 2019, Sviech et al. 2024).

The fungi *Ganoderma lucidum* and *Pleurotus ostreatus* were obtained from the pre-existing collection at the Bioprocesses and Mushrooms Laboratory, both located at the Unicentro. They were grown in potato dextrose agar (PDA) medium and incubated in a biochemical oxygen demand-type growth chamber at 25°C (± 2°C) in a photoperiod of 12 hours of light and 12 hours of darkness for maintenance.

To obtain the treatments, *T. asperellum* was grown in potato dextrose (PD) liquid medium and *G. lucidum* and *P. ostreatus* in fastidious antimicrobial neutralization (FAN) liquid medium, according to the best adaptation of each fungus in its specific growth medium. After 10 days of growth, they were processed individually for 2 minutes in a blender to homogenize the content. After preparing the processed biomass, they were filtered eight times using JP42 blue stripe quantitative filter paper (Quanty) 80 g/m^2, 9 cm diameter, to separate the solid content from the filtered liquid content.

A concentration of 20% of liquid filtrates was used for each treatment. The minimum inhibitory concentration was considered the lowest concentration capable of inhibiting visible growth of the fungus. This concentration was also used for its observed effectiveness in inducing resistance of soybean plants against powdery mildew (Cruz et al. 2019).

The *A. linariae* pathogen used in the research was isolate number 982 from the Laboratory of Biology of Plant Pathogen Populations, at the Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil. It was grown in carrot dextrose agar (CDA) medium and maintained under the same conditions as the other fungi. The induction of sporulation of *A. linariae*, to obtain a spore suspension for *in-vivo* inoculation, occurred after seven days of fungus growth, by injuring (cutting) the mycelium of the fungus, using a scalpel, followed by a short period of time subjected to ultraviolet light (10 minutes of exposure followed by one day of total darkness), to stress induction. The conidia resulting from sporulation were inoculated by water suspension on tomato plants, and the spore concentration used was calibrated to 1×10^6 conidia·mL^{-1}.

Plant material and conduction

The Italian tomato hybrid Grazianni AF 22834 from the company Sakata was used as plant material, with a 115-day cycle, indeterminate growth and compact plants, with medium/low vegetative vigor and short internodes. Sowing took place on August 3, 2022, in expanded polystyrene trays with 128 cells filled with commercial Mecplant substrate. Transplanting was carried out in pots filled with soil, sand, and substrate, in a ratio of 3:1:1. Both soil corrections and fertilization were carried out according to the chemical analysis of the soil performed, aiming to meet the needs of the crop following the recommendations of Alvarenga (2022).

Transplanting was carried out 28 days after sowing, on August 31, 2022, when the seedlings were of an adequate size for the process. Initially, three seedlings were transplanted per pot with subsequent thinning, leaving only two plants per pot to be conducted until the end of the crop life cycle in a single stem, being tutored vertically at 35 days after transplanting (DAT). Other cultural treatments such as disbudding, control of invasive plants, pests and diseases, other than those evaluated in this experiment, were carried out according to the need throughout the crop management and in a way that did not interfere with the application of treatments and evaluations.

Experimental design and treatments

The experimental design used was randomized blocks with five treatments and six replicates, with each plot consisting of four plants. The treatments used were:

- CO: control (water);
- CI: inoculated control (water + inoculum);
- GL: *G. lucidum* biomass filtrate;
- PO: *P. ostreatus* biomass filtrate;
- TA: *T. asperellum* biomass filtrate.

The fungal biomass filtrates obtained for the treatments were applied at a concentration of 20% via foliar spraying on the plant using a CO_2 pressurized backpack sprayer with 0.3 kgf·cm^{-2} with a conical nozzle. All plants received the respective treatment in one single foliar application 28 DAT, and the entire plant was sprayed until the point of runoff was reached. Forty-eight hours later, they were inoculated with the pathogen *A. linariae*, except the control.

Biochemical analyses

To obtain the plant extract used in the enzymatic analyses, leaf samples were obtained between 8 and 9 a.m., the time when the enzymes are most active in the plant (Wang et al. 2022). The samples were placed in an aluminum foil envelope and then frozen in liquid nitrogen, aiming to immediately stop the enzyme activities for later analysis. Plant material sample collections were carried out at 6, 12, 24, 48, 96, 144, and 192 hours after the application of the treatments.

The samples were preserved by keeping the plant material at a temperature of -20°C until the moment of maceration to obtain the extracts to be analyzed later. The enzymatic extraction was adapted according to Balbi-Peña et al. (2014), by liquid nitrogen for maceration in a mortar of approximately 0.2 g of the collected plant material, with subsequent addition of 1% (w/w) of polyvinylpyrrolidone (PVP) and 2 mL of 50 mM potassium phosphate buffer (pH 7.0).

The suspension obtained was then transferred to 2-mL Eppendorf tubes and centrifuged at 15,000 rpm for 30 minutes at 4°C. The supernatant resulting from the process was transferred to 1.5-mL Eppendorf tubes to be stored at -20°C for subsequent enzymatic determination.

Total proteins and enzymatic activity

Total protein quantification was determined according to the Bradford methodology (1976) described by Kruger (2009), with the addition of 50 µL of plant extract to 2.5 mL of Bradford reagent. Using the albumin standard curve, the concentration

of these proteins can be determined, with the absorbance reading performed on a spectrophotometer at a wavelength of 595 nm, and the protein content of the sample demonstrated in mg of protein·g^{-1} of fresh matter.

The activity of the enzyme superoxide dismutase (SOD, EC 1.15.1.1) was determined according to the methodology suggested by Giannopolitis and Ries (1977), which proposes analyzing the enzyme's ability to inhibit the photoreduction of nitrotetrazolium chloride blue (NBT). The determination is performed by adding 50 µL of crude extract to a solution containing 13-mM methionine, 75-µM NBT, 100-mM EDTA, 2-µM riboflavin, and 50-mM sodium phosphate buffer, pH 7.8. The reaction occurs in the presence of light and is stopped in the absence of light.

The evaluation was performed in a spectrophotometer at 560 nm to define a unit of SOD relative to the amount of enzyme necessary to inhibit 50% of the maximum photoreduction of NBT, with the activity of the SOD enzyme in the sample expressed in E.U. min mg of $protein^{-1}$.

The activity of the peroxidase enzyme (POD, EC 1.11.1.7) was performed according to the methodology proposed by Teisseire and Guy (2000). In this methodology, 0.5 mmol·L^{-1} of potassium phosphate buffer (50 mmol·L^{-1} pH 6.5), 0.03 mmol·L^{-1} of enzyme extract, 0.25 mmol·L^{-1} of pyrogallol (1,2,3-benzenetriol) (20 mmol·L^{-1}) and 0.22 mmol·L^{-1} of hydrogen peroxide (H_2O_2) (5 mmol·L^{-1}) are added to a test tube, in a final volume of 1 mL. The test tubes are left in a water bath at 25°C for 5 minutes and then subjected to reading in a spectrophotometer at 430 nm, to determine the formation of purpurogallin. The enzyme activity was calculated using the molar extinction coefficient of 2.47 mmol·L^{-1} cm^{-1}, with the activity expressed in µmol of purpurogallin·min^{-1}·mg^{-1} protein.

The activity of the catalase enzyme (CAT, EC 1.11.1.6) was determined according to the methodology described by Peixoto et al. (1999) with the addition of 50 µL of the enzyme extract and 950 µL of a solution containing 0.05 mol·L^{-1} sodium phosphate buffer, pH 7.0, and 12.5 mmol·L^{-1} H_2O_2. Absorbance readings were performed in a spectrophotometer with a wavelength of 240 nm at 0 and 60 seconds, to verify the reduction in absorbance of each sample. The molar extinction coefficient of H_2O_2 (39.4 mmol·L^{-1} cm^{-1}) was used to calculate the enzyme activity, and the activity was expressed in nmol of H_2O_2 consumed min^{-1}·mg^{-1} protein.

The plant material collected for the other determinations was also used to determine lipid peroxidation at the same times described above. Lipid peroxidation was determined as described by Heath and Packer (1968 *apud* Rama Devi and Prasad, 1998). Then, 0.3 g of fresh frozen leaves were macerated in 5 mL of a solution containing 0.25% thiobarbituric acid (TBA) and 10% trichloroacetic acid (TCA). After the extraction process, the solution was incubated in a water bath at 90°C for 60 minutes and then cooled and centrifuged at 10,000 g for 15 minutes at room temperature (25°C). The supernatant was collected for readings on a spectrophotometer at 560 and 600 nm. The molar extinction coefficient of malondialdehyde (155 mmol.L^{-1}·cm^{-1}) was used for the calculations. The results are expressed in µmol g of fresh·$mass^{-1}$.

Statistical analysis

The data were subjected to analysis of variance (F test) and, subsequently, the means were grouped using the Scott-Knott test at 5% probability. All statistical tests were performed using the SISVAR 5.6 software (Ferreira 2019).

RESULTS AND DISCUSSION

Increasing the efficiency of the antioxidant defense system is essential in the adaptive response to oxidative stress in plants that are in adverse situations such as the presence of pathogens, and it is of great importance to identify elicitors capable of previously activating plant defense.

The plant detects specific pathogens through pathogenic metabolites, which act as biological elicitors capable of triggering defensive responses such as the production of ROS. These responses induced by elicitors are directly involved in the activation of several defense genes that encode a variety of proteins including enzymes, secondary metabolites, and PRPs (Appu et al. 2021).

ROS produced by a stimulus are essential in signaling related to the plant's defense response to pathogens, and their excess can cause harmful effects on cells and their components, demonstrating the importance of antioxidants as molecules capable of inhibiting reactions of these free radicals, delaying or preventing damage by avoiding the oxidation process (Dumont and Rivoal 2019, Dumanović et al. 2021).

To understand the possible physiological responses of treatment with fungal biomass filtrates with potential for use as biocontrol agents (BCA), the plants were treated, and their enzymatic activity was monitored after application of the treatments and inoculation of the pathogen *A. linariae* to evaluate the stress process and the antioxidant response of these plants.

According to the results observed in Table 1, and before inoculation of the pathogen, the *G. lucidum* and *T. asperellum* filtrates presented the highest initial enzymatic responses for the activities of the SOD and CAT enzymes 6 hours after the application of the treatments in both experiments, demonstrating that they are capable of rapidly activating the plant's defense system without compromising the cells due to excess of ROS and their lipid peroxidation damage, considering that these treatments presented the lowest values of this parameter at the same time.

Table 1. Superoxide dismutase (SOD, U min^{-1}·mg $protein^{-1}$), catalase (CAT, U min^{-1}·mg $protein^{-1}$), peroxidase (POD, µmol purpurogallin·min^{-1}·mg $protein^{-1}$), and lipid peroxidation (TBARS, µmol g fresh·$mass^{-1}$) enzyme activity of tomato plants after 6, 12 and 24 hours after application of treatments in experiments 1 and 2*.

Treatments	6 h				12 h				24 h			
	SOD											
	1		2		1		2		1		2	
CO	12.817	b	14.735	c	12.899	b	12.035	b	16.440	a	13.027	c
GL	22.876	a	16.853	b	10.941	b	12.312	b	9.158	b	13.198	c
PO	12.855	b	13.626	d	16.035	a	13.679	a	17.485	a	14.842	b
TA	21.584	a	21.223	a	16.890	a	12.123	b	15.879	a	22.831	a
Treatments	**CAT**											
	1		2		1		2		1		2	
CO	0.131	c	0.109	c	0.100	c	0.139	b	0.145	a	0.190	a
GL	0.169	a	0.199	a	0.151	a	0.129	a	0.066	c	0.084	c
PO	0.153	b	0.071	d	0.117	b	0.048	c	0.100	b	0.148	b
TA	0.168	a	0.120	b	0.101	c	0.136	a	0.101	b	0.086	c
Treatments	**POD**											
	1		2		1		2		1		2	
CO	0.941	b	1.980	b	0.937	b	2.166	c	2.588	a	1.354	c
GL	1.303	a	2.225	a	0.997	b	3.402	a	1.349	c	1.100	d
PO	1.199	a	1.652	d	1.015	b	2.782	b	2.036	b	2.699	a
TA	0.976	b	1.878	c	1.727	a	2.818	b	2.681	a	1.638	b
Treatments	**LP**											
	1		2		1		2		1		2	
CO	6.712	a	7.412	a	6.076	ns	6.129	ns	7.151	ns	7.062	ns
GL	6.276	b	6.417	b	5.889		6.184		6.971		7.010	
PO	7.030	a	6.768	b	6.061		6.205		6.838		7.018	
TA	5.937	b	6.661	b	6.349		5.731		6.611		7.438	

CO: control (distilled H_2O); GL: *Ganoderma lucidum* biomass filtrate 20%; PO: *Pleurotus ostreatus* biomass filtrate 20%; TA: *Trichoderma asperellum* biomass filtrate 20%; *means followed by distinct letters differ by the Scott-Knott test at the 5% probability level ($p < 0.05$).

According to Table 1, the GL treatment maintained high activity of the SOD and CAT enzymes 12 hours after application, in addition to the highest activity of the POD enzyme in experiment 2, while the TA treatment presented high enzymatic activity of the SOD and POD enzymes in experiment 1 and higher activity of the CAT enzyme in experiment 2, and the

PO treatment presented the highest averages of the SOD enzyme, demonstrating the activity of the signaling process in the plants that received these treatments. The lipid peroxidation values obtained at this time did not differ statistically between any of the treatments tested.

Pathogenic toxins are taken up by the host, and they induce chlorosis by disrupting photosynthesis and trigger a hypersensitive response through the uncontrolled accumulation of ROS (Schmey et al. 2024). The superoxide anion radical ($O_2^{\cdot-}$) is the first ROS generated by the cell in the oxidative stress process, triggering a cascade of reactions and, through reactions catalyzed by metals, the SOD enzyme quickly performs the dismutation of this free radical, forming O_2, H_2O, and another ROS, hydrogen peroxide (H_2O_2), serving as a substrate for the CAT and POD enzymes.

As observed in the results of this research, the GL and TA treatments stood out for their agility in activating the plant signaling enzymatic apparatus, especially in the first 12 hours after their application, reinforcing the importance of the action of these enzymes for the formation of post-formed components related to plant defense against pathogens. The CAT enzyme interacts with the signaling pathways of jasmonates, regulators of plant responses associated with defense against necrotrophic and herbivorous pathogens, and salicylates in defense against biotrophic pathogens, playing a crucial role in modulating the balance between the signaling pathways of these regulators (Huang et al. 2023).

Secondary metabolite biosynthesis is a crucial defense response to necrotrophic pathogens. *Alternaria* spp. produces over 70 described secondary metabolites that are not precisely elucidated, but there is an indication that the diversion of mitochondrial electrons to generate ROS plays a significant role in the infection process (Sadeghi et al. 2022).

In typical necrotrophic pathogenesis, such as observed in *A. linariae*, cell wall-degrading enzymes break down physical barriers to access host nutrients and activate the plant's immune system strongly enough to trigger a hypersensitive response, leading to programmed cell death (Mengiste 2012).

The POD enzyme, in turn, is involved in critical metabolic processes of the plant cell wall, catalyzing reactions such as suberization, auxin metabolism, and crosslinking of cell wall polymers, also contributing to lignification, an important mechanism in the structural defense response of the host plant against pathogen infections, reinforcing the physical and chemical barrier to limit the spread of the invading agent (Appu et al. 2021).

After 24 hours of application, a significant change in the enzymatic response of the GL treatment was observed, and the enzymes SOD, CAT, and POD exhibited the lowest activities at this time, evidencing that the signaling process and enzymatic balance of the plants had been reached. The enzymatic activity of the plants at this same time demonstrates that the TA treatment still presented significant activity in the values of the SOD enzyme, evidencing that the stress of this treatment was still present for a long period.

The pathogen was inoculated 48 hours after the application of the treatments, and as observed in Table 2, the GL, PO, and TA treatments presented the highest activities of the SOD enzyme, while the CI treatment presented the lowest activity of this enzyme at the same time.

Two days after pathogen inoculation, the GL treatment showed an increase in the activity of the POD enzyme in both experiments, demonstrating the efficiency of this treatment in the defense against plant diseases, acting as an effector by expressively inducing the activation of this enzyme responsible for a series of defense-related processes, including the hypersensitive response, production of phytoalexins, lignification, cross-linking of phenolics and glycoproteins, and suberization (Prasannath 2017, Thakker et al. 2013).

Zhang et al. (2019) observed that the use of *G. lucidum* polysaccharide (GLP) can induce systemic resistance in cotton plants inoculated with *Fusarium oxysporum*, increasing the expression of genes related to plant defense, as well as increasing the activity of the POD and SOD enzymes, corroborating the results found in this study.

At 96 and 144 hours after inoculation, it was noted that the CO and CI treatments showed high activity of the SOD and CAT enzymes, an effect opposite to that observed in the other treatments. This behavior can be attributed to the high incidence of disease in these plants resulting from the inoculation and the presence of secondary inoculum in the control plants that did not receive prior treatment to activate the defenses. The application of the GL, PO, and TA treatments was able to reduce the values of lipid peroxidation up to 144 hours after inoculation in experiment 2, demonstrating that all were able to reduce the deleterious effects caused by the presence of the pathogen in these plants, in addition to presenting the same behavior for the GL treatment in both experiments 96 hours after inoculation.

Table 2. Superoxide dismutase (SOD, U min^{-1}·mg $protein^{-1}$), catalase (CAT, U min^{-1}·mg $protein^{-1}$), peroxidase (POD, µmol purpurogallin·min^{-1}·mg $protein^{-1}$), and lipid peroxidation (TBARS, µmol·g fresh $mass^{-1}$) enzyme activities of tomato plants after 1, 48, 96, and 144 h after inoculation with *Alternaria linariae* in experiments 1 and 2*.

Treatments	1 h				48 h				96 h				144 h			
	SOD															
	1		2		1		2		1		2		1		2	
CO	18.02	b	9.50	c	18.73	a	7.14	b	15.34	d	10.51	b	21.35	b	16.78	b
CI	13.73	c	7.54	d	20.44	a	5.10	c	35.22	a	12.68	a	28.26	a	20.87	a
GL	23.54	a	12.91	a	14.63	b	7.84	a	23.00	c	10.43	b	16.37	c	11.50	d
PO	24.43	a	10.94	b	20.01	a	7.23	b	22.18	c	10.98	b	28.03	a	15.22	c
TA	24.66	a	10.29	b	14.96	b	7.94	a	26.93	b	11.15	b	19.71	b	17.77	b
Treatments	CAT															
	1		2		1		2		1		2		1		2	
CO	0.11	c	0.05	d	0.15	a	0.11	a	0.22	a	0.10	b	0.15	a	0.18	b
CI	0.22	a	0.15	a	0.13	b	0.12	a	0.22	a	0.11	a	0.16	a	0.27	a
GL	0.09	d	0.07	c	0.08	d	0.10	b	0.12	c	0.05	e	0.14	b	0.06	d
PO	0.16	b	0.08	b	0.10	c	0.11	a	0.20	b	0.06	d	0.15	a	0.08	c
TA	0.12	c	0.06	d	0.08	d	0.07	c	0.21	b	0.08	c	0.09	c	0.05	d
Treatments	POD															
	1		2		1		2		1		2		1		2	
CO	1.67	b	0.65	c	0.86	c	0.72	e	0.86	d	0.63	d	1.72	b	1.57	b
CI	1.32	c	0.81	b	1.01	b	1.18	c	1.38	c	0.59	d	1.98	a	1.51	b
GL	1.78	b	0.69	c	1.11	a	1.67	a	1.66	b	1.68	a	1.34	d	1.25	c
PO	1.76	b	1.10	a	1.22	a	1.35	b	1.71	b	1.17	b	1.04	e	2.13	a
TA	2.03	a	0.87	b	1.20	a	1.00	d	1.96	a	0.80	c	1.53	c	1.00	d
Treatments	LP															
	1		2		1		2		1		2		1		2	
CO	6.29	ns	6.50	a	6.55	ns	4.77	a	5.76	ns	6.23	a	6.05	a	5.40	a
CI	6.16		6.36	a	6.81		4.86	a	5.64		6.39	a	6.09	a	5.42	a
GL	6.40		5.92	b	6.94		4.04	b	6.35		5.65	b	5.35	b	4.68	b
PO	6.55		5.74	b	7.05		4.41	b	5.95		5.45	b	6.03	a	4.45	b
TA	6.21		5.39	b	6.43		4.39	b	5.76		5.84	b	5.86	a	5.08	b

CO: control (distilled H_2O); GL: *Ganoderma lucidum* biomass filtrate 20%; PO: *Pleurotus ostreatus* biomass filtrate 20%; TA: *Trichoderma asperellum* biomass filtrate 20%; *means followed by distinct letters differ by the Scott-Knott test at the 5% probability level ($p < 0.05$).

Fungal species such as those of the *Trichoderma* genus are well known for acting systemically in activating plant defenses, but in addition to this, other species such as *G. lucidum* are capable of inducing resistance to pathogens by activating related genes and *P. ostreatus* presenting great antibacterial and antifungal effects (Sood et al. 2020, Seo and Choi 2021, Suliaman et al. 2021, Yang et al. 2022).

In the indirect form of disease control by inducing resistance, BCAs are capable of inducing plant-mediated responses, allowing the plant to react more quickly and efficiently after subsequent pathogen attack (De Lorenzo and Cervone 2022). This study shows that the use of BCAs, especially *G. lucidum* biomass filtrate 20%, can be used as an alternative to reduce the use of fungicides against *A. linariae* (Fig. 1).

The results observed in this work, added with the results of parameters such as antagonistic potential (*in vitro*), disease severity, crop development, and photosynthetic parameters observed by De Morais et al. (2024), demonstrated that, even at different intensities, these filtrates are capable of inducing plant enzymatic defenses, reducing disease severity in plants, and can be considered promising for their use as plant elicitors in agriculture.

Figure 1. Symptoms of *Alternaria linariae* on tomato leaves. (a) *Ganoderma lucidum* biomass filtrate 20% treatment and (b) inoculated control 33 days after transplanting; (c) *Ganoderma lucidum* biomass filtrate 20% treatment and (d) inoculated control 38 days after transplanting.

CONCLUSION

The identification of new organisms is a critical step in the development of commercial biocontrol products and requires screening methods to evaluate candidates for BCAs. In this work, it was demonstrated that the use of fungal biomass filtrate from *G. lucidum* contributes to the induction of the defense system in tomato plants used in this assay against the pathogen *A. linariae* and has great potential as a complementary alternative to pesticides in crop protection.

Future research is essential to elucidate the specific bioactive compounds present in fungal biomass filtrates that are responsible for inducing resistance in tomato plants. Understanding these components and their mechanisms of action will enable the development of targeted and efficient bioproducts. This knowledge could contribute significantly to sustainable agriculture by reducing dependence on chemical fungicides.

CONFLICT OF INTEREST

Nothing to declare.

AUTHORS' CONTRIBUTION

Conceptualization: Morais, A. F., Sapelli, K. S., Schwan-Estrada KRF, Mazaro SM and Faria, C. M. D. R.; **Data curation:** Morais, A. F. **Formal Analysis:** Morais, A. F.; **Writing – original draft:** Morais, A. F. and Sapelli, K. S.; **Writing – review & editing:** Morais, A. F., Sapelli, K. S., Schwan-Estrada KRF, Mazaro SM and Faria, C. M. D. R.; **Project administration:** Morais, A. F. and Sapelli, K. S.; **Final approval:** Morais, A.F.

DATA AVAILABILITY STATEMENT

The datasets generated during the current study are available from the corresponding author on reasonable request.

FUNDING

Conselho Nacional de Desenvolvimento Científico e Tecnológico
Grant No.: 401055/2019-1

ACKNOWLEDGMENTS

Not applicable.

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