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Maize protection against Bipolaris maydis using Lentinula edodes, Aloe vera and Acibenzolar-S-Methyl

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Abstract:	Maize (Zea mays) is the most commonly produced grain worldwide. One of the factors that can lead to a decrease in productivity is the Southern Leaf Blight caused by Bipolaris maydis. This is common in the main producing regions and can promote losses of up to 70% under suitable conditions. A few genotypes are resistant to this fungus, and there is no record of fungicides for the disease in Brazil. In order to search for alternative control measures with lower environmental impacts, the objective of this study was to investigate the use of polysaccharide fractions extracted from shiitake or aloe, as well as acibenzolar-S-methyl (ASM), as potential inductors of resistance in maize against B. maydis. Tests were conducted with maize plants (hybrids P1630H and BM3063) to evaluate the effects of the products on reducing disease severity, inhibiting pathogen germination, and activating guaiacol peroxidase (GPX) and phenylalanine ammonia-lyase (PAL) activities. Aloe and ASM were not efficient to control the disease. Fraction PS1 from shiitake reduced the disease severity in both hybrids by approximately 60%. Meanwhile, fraction PS2 was efficient only in hybrid BM3063, where it promoted an increase in PAL activity. However, these fractions did not affect the spore germination of B. maydis. Therefore, the polysaccharide fractions from shiitake have the potential to control Southern Leaf Blight (B. maydis) in maize probably through the activation of plant defense mechanisms.
Response to Reviewers:	Manuscript Number: JPDP-D-23-00314 Title: Maize protection against Bipolaris maydis using shiitake, aloe and ASM Dear Editor, We would like to thank the reviewers for their questions and suggestions, which contributed to the improvement of the manuscript. Below are our answers. The changes we have made to the new manuscript version are highlighted there in yellow. Best regards, The authors Reviewer 1: 1) Cite recent references (line 37 of the original version). A: The reference Singh et al. (2021) was added (line 38 of the latest version). 2) Cite recent references (line 42 of the original version). A: The reference Malik et al. (2018) was added (line 43 of the latest version). 3) Cite recent references (lines 47-48 of the original version). A: The references Kashyap et al. (2022) and Schauffler et al. (2022) were added. (lines 48-49 of the latest version) 4) Add the properties of polysaccharides against fungal foliar plant diseases, like maydis leaf blight, turcicum leaf blight, leaf spot of rice etc . A: We did not find studies on the use of polysaccharides against the mentioned diseases. 5) Update with recent references (line 58 of the original version).

	A: The references Kaur et al. (2016) and Oliveira et al. (2019) were added (lines 64-66 of the latest version). 6) Highlight the properties of acibenzolar- S-methyl (ASM). A: The information has been added (lines 52-55 of the latest version). 7) Change “control” for “manage”. A: The change has been made (line 71 of the latest version). 8) Mention primer details and their reaction cycle in precise manner. A: The details were added. (lines 87-93 of the latest version) 9) Mention dark and light hours. A: The dark/light hours were mentioned. (Lines 95, 98 and 100 of the latest version). 10) Change “Inoculum” for “inoculums”. A: The change has been made (line 101 of the latest version). 11) Change “destilled” for “distilled”. A: The change has been made (line 118 of the latest version). 12) Write the same figure everywhere, 104 conidia mL-1 is a standard. A: In this experiment we used a concentration of 1x103 conidia.mL-1 (line 141 of the latest version). 13) One place you wrote 104 conidia mL-1 another place103 conidia mL-1, Why? A: We used a concentration of 1x103 conidia.mL-1 for pathogen inoculation, except in the spore germination experiment. In this case, a concentration of 1x104 conidia.mL-1 was used to ensure an adequate quantity of spores for evaluation under an optical microscope. 14) Change “a” for “an”. A: The change has been made (line 169 of the latest version). 15) Add the recent references. A: The reference Schauffler et al. (2022) was added (line 177 of the latest version). Reviewer 3: 1) In the title the ASM should be written in the full name not an abbreviation in the title and even the shiitake and aloe prefer to have their scientific names written if known. A: The full name of acibenzolar-s-methyl and the scientific names of Lentinula edodes and Aloe vera were provided in the title (lines 1 and 2 of the latest version). 2) Line 84, The flask should be a vial rather than the flak why the flask A: The change has been made (line 96 of the latest version). 3) The use of solid statistics such as the Generalized Linear model has not been used for the analysis such as logistic and log-linear analyses rather than Graph Pad Prism. A: The statistical analyses were performed using parametric tests through the software Statistica 6.0. The information was corrected (line 196 of the latest version). 4) In the spore germination experiment how many replicates were used or the number of spores. A: The experiment was conducted with five replicates per treatment and we observed 100 spores from each sample. The information has been added (lines 158-160 of the latest version). 5) In Figure 1, how we can distinguish if they are statistically different from each other because the letters are not written on it. A: The letters indicating that there was no significant difference between the treatments were added to the figure 1. 6) The SD indicates that the data points are very spread out as it is very high which is not good and it is better to find the error bars rather than the standard deviation for

	graphical illustration. A: The standard errors of the means were added (Figures 4 and 5).

Maize protection against *Bipolaris maydis* using *Lentinula edodes*, *Aloe vera* and Acibenzolar-S-Methyl

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Abstract

Maize (*Zea mays*) is the most commonly produced grain worldwide. One of the factors that can lead to a decrease in productivity is the Southern Leaf Blight caused by *Bipolaris maydis*. This is common in the main producing regions and can promote losses of up to 70% under suitable conditions. A few genotypes are resistant to this fungus, and there is no record of fungicides for the disease in Brazil. In order to search for alternative control measures with lower environmental impacts, the objective of this study was to investigate the use of polysaccharide fractions extracted from shiitake or aloe, as well as acibenzolar-S-methyl (ASM), as potential inductors of resistance in maize against *B. maydis*. Tests were conducted with maize plants (hybrids P1630H and BM3063) to evaluate the effects of the products on reducing disease severity, inhibiting pathogen germination, and activating guaiacol peroxidase (GPX) and phenylalanine ammonia-lyase (PAL) activities. Aloe and ASM were not efficient to control the disease. Fraction PS1 from shiitake reduced the disease severity in both hybrids by approximately 60%. Meanwhile, fraction PS2 was efficient only in hybrid BM3063, where it promoted an increase in PAL activity. However, these fractions did not affect the spore germination of *B. maydis*. Therefore, the polysaccharide fractions from shiitake have the potential to control Southern Leaf Blight (*B. maydis*) in maize probably through the activation of plant defense mechanisms.

Keywords: induced resistance, alternative management, shiitake, ASM.

Introduction

Maize (*Zea mays*) is currently the most commonly produced grain worldwide, with an average production of 1.1 billion tons, owing to its extensive use in the production of flour, oils, animal feed, and ethanol (Erenstein et al., 2022).

This production can be reduced in fields affected by diseases such as Southern Leaf Blight, caused by the fungus *Bipolaris maydis* (Nisik.) Shoemaker, widely disseminated in the producing regions. It causes significant losses of up to 70% in cultivars from subtropical and temperate regions (Ali et al. 2011; Singh et al. 2021).

Currently, in Brazil, the main control measure for *B. maydis* is crop rotation because there are no fungicides registered for the fungus and few genotypes are resistant. In this context, there is a need for research technologies that contribute to disease management within new sustainable trends in the development of phytosanitary products (Pimentel and Burgess, 2013; Malik et al. 2018).

Induced resistance is a phenomenon carried out intentionally, aiming to activate plant defense mechanisms through the application of biotic or abiotic substances that can be recognized by plant cells. Plant defense mechanisms are classified as structural or biochemical and are subdivided into constitutive or induced as a result of interaction with the pathogen and activation by their elicitor molecules (Thakur and Sohal, 2013; Kashyap et al. 2022; Schauffler et al. 2022).

Abiotic elicitors such as acibenzolar-S-methyl (ASM), an analog of salicylic acid, are registered against some diseases and are being researched as an alternative measure for others. The defense responses triggered by ASM are associated with expression of genes from the salicylic acid pathway (Darolt et al., 2020), the increase in pathogenesis-related proteins (PR-proteins), and the accumulation of reactive oxygen species (ROS) and consequent hypersensitivity reaction (Zuluaga, et al. 2013).

Biotic elicitors obtained from sources such as algae, plants, and microorganisms can be represented by polysaccharides such as acemanan, pectin, glucans, and chitin (Luiz et al, 2017; Rodrigues et al., 2021). Polysaccharides obtained from aloe leaves have been shown to be effective in reducing the severity of diseases, such as tomato bacterial spot (*Xanthomonas gardneri*) and strawberry angular spot (*X. fragariae*), and in activating defense enzymes, including peroxidase, superoxide dismutase, polyphenol oxidase, glucanase, and catalase (Luiz et al. 2015; Luiz et al. 2017). The aqueous extract of *Lentinula edodes* (shiitake mushroom) reduced the severity of *Exserohilum turcicum* and *Colletotrichum sublineolum* in sorghum cultivars (Piccinin et al. 2010), *C. lindemuthianum* on common bean (Oliveira et al. 2019) and *X. campestris* pv. *campestris* in tomato (Kaur et al. 2016). Meanwhile, the aqueous extract from spent substrate suppressed lesions caused by *Pyricularia oryzae* in rice, causing the accumulation of phytoalexins and changing plant hormone levels (Ishihara et al. 2019). However, only a few studies have been conducted with shiitake polysaccharides.

Therefore, the objective of the present study was to investigate the efficiency of aloe and shiitake polysaccharides, as well as ASM, to manage the disease caused by *B. maydis* in maize and elucidate their modes of action.

Material and Methods

Plant and Pathogen

The experiments were conducted inside a greenhouse using two maize commercial hybrids: P1630H, simple hybrid (DuPont Pioneer, Johnston, IA, USA) and BM3063, triple hybrid (Biomatrix, Rio Claro, Brazil) both considered susceptible to *B. maydis*. Four seeds were sown in plastic pots (2 liters), containing a mixture of the

commercial substrate Solo Fertilizado and peat substrate (2:1). Thinning was conducted after approximately 7 days, and two seedlings remained in each pot.

The isolate of *B. maydis* used in the present study, code MANE 188, is deposited in the Plant Pathology Laboratory (UFSC). It was isolated from symptomatic leaf samples collected in an experimental farm in Florianópolis,SC, Brazil, as described by Da Silva (2018). The molecular identification was carried out by the company Helixxa Genomic Services (Florianópolis, Brazil) with primers from the ITS1-ITS2 region according to De Hoog and Guerrits Van Den Ende (1998). For PCR amplification 40 cycles were performed: 94 °C for 1 min (delay); 94 °C for 1 min (denaturation), 58 °C for 1 min (annealing), 72 °C for 2 min (extension). The fragments were sequenced with ABI 3500 Genetic Analyser (Applied Biosystems, Waltham, MA, USA) using BigDye Terminator v 3.1 Cycle Sequencing kit. The sequences were aligned and compared with the NCBI database.

B. maydis was kept in Petri dishes with PDA (potato-dextrose-agar), incubated in a growth room at 25 °C with photoperiod of 12 h light / 12 h dark for 15 days. After that period, four mycelium discs were transferred to a 125 mL Erlenmeyer vial containing 30 g of sorghum grains and 30 mL of distilled water, previously autoclaved twice for 30 min in a 24 h interval. Subsequently, the vials were incubated (25 °C – 12 h light / 12 h dark) during 10 days for the complete colonization of the sorghum grains and incubated in a humid chamber (25 °C – 12 h 12 h light / 12 h dark) for more 3 days to induce the fungus to sporulation. For the preparation of the inoculums suspension, the colonized sorghum grains were transferred to a 50 mL falcon tube, until 1/3 of the volume was completed, and subsequently 20 mL of sterile distilled water was added. The tubes were vortexed, the suspension filtered and its concentration adjusted, with the aid of a Neubauer chamber.

Inducers

Residual stipes from shiitake mushroom, provided by Professor Sérgio Florentino Pascholati (ESALQ/USP), were processed with water 1:10 (m/v) and autoclaved for 30 min at 120 °C. After cooling, the mixture was filtered, thus obtaining the aqueous crude extract which was submitted to precipitation with 92% ethanol in the proportion of 1:1 (v/v), at 4 °C for 48 h. The precipitated phase was collected and constituted the polysaccharide fraction of shiitake (PS1), while the supernatant was again submitted to precipitation, using 92% ethanol but in the proportion of 1:3 (v/v), kept at 4 °C for 48 h. After a second centrifugation, the collection of the precipitated phase was performed, obtaining PS2. Both polysaccharide fractions, PS1 and PS2, were dried at 45 °C until constant weight. The dry material was crushed with the aid of an analytical mill and stored at -20 °C (Chihara et al. 1970). For application in the plants, the shiitake polysaccharides were resuspended in distilled water.

The polysaccharide of aloe was obtained from aloe leaves provided by the company Naturama Sucos Integrais do Brasil Ltda, Paulo Lopes, SC. The reserve parenchyma from aloe leaves was processed in an industrial blender, added with 92% ethanol in the proportion of 1:3 (v/v) and stored at 4 °C for 48 h. Subsequently, the precipitated showing white color and fibrous aspect (aloe polysaccharide - AP) was collected. It was submitted to drying at 45 °C until reaching constant weight, crushed with the aid of an analytical mill and stored at -20 °C. For use in the assays, the aloe polysaccharides were resuspended in distilled water, submitted to constant agitation for 15 min at 3,600 rpm and incubated for 30 min at 100 °C as a standard preparation. The polysaccharide suspension was incubated at 8 °C for 24 h before application to the plants (Luiz et al. 2015).

Acibenzolar-S-methyl (ASM) was obtained from the commercial product Bion® 500WG (Syngenta) which contains 50% of active ingredient.

Maize protection against *Bipolaris maydis*

Maize plants (hybrids P1630 and BM3063) were sprayed with ASM at 25, 50 and 100 $mg.L^{-1}$, aloe polysaccharides at 0.5, 1.5 and 3 $mg.mL^{-1}$ or shiitake polysaccharides at 3 and 5 $mg.mL^{-1}$, when reached the stage V4. As a control, the plants were sprayed with distilled water. The experimental design was completely randomized with six replicates per treatment, and the experimental unit was represented by a pot containing two plants.

After a time interval of the application of the products (3 or 5 days), the plants were inoculated by spraying the pathogen *B. maydis* (1×10^3 conidia.mL^{-1}) to the pont of run-off and remained under humid chamber during 24 h. The disease severity was evaluated at 5 and 10 days after inoculation (DAI) in the third and the fourth leaves of each plant with the aid of the diagrammatic scale established by Marcos et al. (2015).

Spore germination

Maize plants (hybrids P1630 e BM3063) at V4 stage were sprayed with destilled water or shiitake polysaccharides at 3 $mg.mL^{-1}$ (fractions PS1 and PS2). The third leaves from four plants for each treatment were detached at 3 days after the spraying and their middle portions were placed in gerbox-type boxes containing moistened filter paper (two sheets per box). On the abaxial surface of each leaf, 8 drops of 20 µL of spore suspension (1×10^4 conidia.mL^{-1}) were pipetted. The boxes were sealed with plastic film and placed inside a BOD-type chamber at a temperature of 25 °C and a photoperiod of 12 h. After 24 h, leaf discs were collected and placed on bleached solution composed by

ethanol:acetic acid (3:1) and the solution was changed each 24 h during 3 days (Daudi and O'Brien, 2012). After this period, the discs were placed on conservation solution containing ethanol, acetic acid and glycerol (3:1:1) until the analyses. The number of germinated spores was quantified with the aid of an optical microscope (Alltion®). The experiment was conducted with five replicates per treatment and 100 spores from each sample were observed.

Biochemical defenses

An experiment was conducted in a completely randomized design with 5 replicates. Thus, the PS1 and PS2 fractions, at a dose of 3.0 $mg.mL^{-1}$, were applied to the two maize hybrids, when they were in V4, at 3 days before inoculation with the pathogen (10^3 $conidia.mL^{-1}$).

The leaf samples were collected immediately before the application of inducers (day 0), and also at 3, 4 and 6 days after application (corresponding to 0, 1 and 3 days after the inoculation). The third and fourth leaves of a single plant were considered as an experimental unit. The samples were collected and stored at -80ºC until the moment of analysis.

Leaf samples (500 mg) were ground with liquid nitrogen and the obtained powder resuspended in 6.0 ml of extraction buffer (50 mM sodium phosphate, pH 5.2, for peroxidases or 25 mM sodium borate, pH 8.8, for phenylalanine ammonia lyase) containing 1 mM EDTA and 0.5% polyvinylpyrrolidone. The samples were centrifuged at 15 000 × *g* for 30 min at 5 ºC and the collected supernatant was considered the protein extract (Schauffler et al. 2022).

The guaiacol peroxidase activity was determined from the addition of 5 µL of the protein extract to 155 µL of extraction buffer containing 1% guaiacol (v/v) and 0,3%

hydrogen peroxide (v/v). The reaction was conducted for 5 minutes at 30ºC in a 96-well polysterene microplate, and optical density values (OD) at 470 nm were recorded every 30 seconds (Hammerschmidt *et al.,* 1982). The results were expressed in OD_{470nm}.mg $protein^{-1}.min^{-1}$.

The phenylalanine ammonia lyase activity was determined from the addition of 25 µL of the protein extract to 475 µL of sodium borate buffer (100 mM, pH 8,8), containing phenylalanine (50 mM). The reaction was conducted for 1 h at 40ºC in Eppendorf tubes, and then stopped by adding 5 N HCl. Next, 300 µL of distilled water was added to the tubes and optical density values at 290 nm were recorded (FALCÓN et al., 2008). The results were expressed in nmol of trans-cinamic acid.mg $protein^{-1}.min^{-1}$. The total protein content was quantified by the Bradford method (1976).

Statistical analysis

Data were submitted to variance analysis (ANOVA) and to tests of separation of means, when the ANOVA was significant. The disease severity means were analyzed by Tukey's test and the enzyme activities by the Dunnet's test. The analyses were performed in the Statistica 6.0 software.

Results

Protection of maize plants

Acibenzolar-S-methyl and aloe polysaccharides did not reduce the severity of the Bipolaris spot in maize plants from the P1630H hybrid (Figure 1) and from the BM3063 hybrid (data not shown) 10 days after inoculation, regardless of the dose.

In contrast, shiitake polysaccharide fractions reduced the severity of the disease in both hybrids. For the P1630H hybrid, the PS1 fraction was always more efficient than the PS2 fraction, promoting a significant reduction of the disease when applied 3 days before inoculation (Figure 2). For the hybrid BM3063, both fractions, PS1 and PS2, applied at 3 and 5 mg · mL^{-1} at 3 or 5 days before inoculation, reduced the disease severity for both evaluation times (by around 65%) (Figure 3).

Spore germination

The shiitake polysaccharide fractions (PS1 and PS2) did not significantly alter the number of *B. maydis* spores that have germinated on maize leaves. An average of 12 spores germinated per cm^2 of leaf area was observed (Table 1).

Evaluation of enzymatic activity

The PS1 fraction did not change the enzymatic activity of GPX and PAL in the hybrid P1630H (Figures 4A and 4B) neither in the hybrid BM3063 (Figures 5A and 5B). However, the PS2 fraction promoted a significant increase (60%) in the enzymatic activity of GPX at 3 days after its application (Figure 4C), as well as a significant increase (200%) in the enzymatic activity of PAL 3 days after P1630H inoculation with *B. maydis* (Figure 5D). In hybrid BM3063, PS2 fraction did not change the GPX activity (Figure 5C), but promoted a significant increase (100%) in PAL activity at 3 days after the inoculation with *B. maydis* (Figure 5D).

Discussion

Activation of the plant defense mechanisms for biotic or abiotic elicitors has been widely studied as alternative management because of their reduced environmental

impacts. In systemic acquired resistance, salicylic acid (AS) plays a fundamental role in signaling, as well as in local and systemic defense activation and potentially in the regulation of cell death (Steiner and Schönbeck, 1995; Dempsey et al. 1999).

Acibenzolar-S-methyl (ASM) is a synthetic analog of AS that directly influences this defense signaling pathway, promoting an increase in reactive oxygen species (ROS), the hypersensitivity reaction (HR), and the expression of PR proteins in treated plants (Zuluaga, et al. 2013). Currently, this active principle is present in the composition of commercial products such as Bion®, registered in Brazil for 29 diseases, two of them involving monocots: downy mildew in onion, caused by *Peronospora destructor* and powdery mildew in wheat, whose causal agent is the fungus *Blumeria graminis* f. sp. *tritici*.

In maize, Morris et al. (1998) reported the induced resistance to mildew (*Peronosclerospora sorghi*) when seeds were previously treated with ASM. Under field conditions, ASM did not reduce the severity of cercosporiosis (*Cercospora* spp.) (Barros 2011). In the present study, ASM was not efficient against *B. maydis* in maize.

Plant colonization influences the activated signal transduction pathway, as shown in *Arabidopsis*, with biotrophic pathogens inducing defense responses signaled by the salicylic acid pathway. Meanwhile, necrotrophic pathogens signal the jasmonic acid and ethylene pathways (Halim et al. 2006). In maize, this type of response may also occur because the application of ASM, a product related to the salicylic acid pathway, was inefficient in the presence of *B. maydis*, a necrotrophic fungus. In line with these data, Ziemann et al. (2018) observed an increase in the susceptibility to necrotrophic pathogens (*Botritys cinerea*) in maize after the application of an apoplastic peptide (Zip1) extracted from leaves previously treated with AS.

The polysaccharide fraction of aloe predominantly contains acemanan and pectin, which can function as elicitors similar to damage-associated molecular patterns (DAMPs). These are generated by the action of microbial enzymes on the plant cell wall. Oligogalacturonides have been reported to activate defense responses, such as ROS production and the accumulation of phytoalexins and defense proteins (Minjares-Fuentes et al. 2018; Hou et al. 2019).

Aloe polysaccharides (AP) have recently demonstrated efficiency in inducing resistance against bacterial pathosystems, such as *Xanthomonas gardneri* (tomato) and *Xanthomonas fragariae* – strawberry, activating enzymes, such as peroxidase, superoxide dismutase, polyphenol oxidase, and phenylalanine ammonia lyase, with additional accumulation of hydrogen peroxide and an increase in the levels of flavonoids (Luiz et al. 2015; Luiz et al. 2017).

The results of AP against bacteriosis contrasted with those obtained for the fungus *B. maydis*, given this polysaccharide did not reduce disease severity efficiently. Acemanan has a low solubility and hydrophobicity (Moreira 2008; Chokboribal et al. 2015), making it difficult for it to cross the cell wall, which is composed of 60% water (Vorwerk et al. 2004; Pettolino et al. 2012). In addition to not reaching the membrane receptors to activate maize defense responses, polysaccharides remain on the leaf surface and can serve as a source of sugar for *B. maydis*, a necrotrophic pathogen. This explains the increase in disease severity observed in one of the experiments.

The shiitake polysaccharide fractions used in the present study (PS1 and PS2) were effective in reducing the severity of Southern Leaf Blight and activating defense enzymes in maize, especially PS2. Chihara et al. (1970) characterized as lentinan (long-chain β-1,3-glucan) a fraction of shiitake precipitated with ethanol in the proportion of 3:1, suggesting that this compound could be part of the fraction PS1. The PS2 fraction

could represent glucans or other sugars of low molecular size because it was precipitated using a greater proportion of ethanol than the first fraction (Xu et al. 2014).

Balmer et al. (2013) reported successful cases of induced resistance in monocots, finding four receptor proteins in rice (FLS2, CEBiP, CERK1, and Xa21) capable of recognizing microbial patterns such as flagellin, chitin, and amino acids. For maize, there is only one hypothesis that a receptor protein is related to the volatile compounds released during herbivory processes.

Wanke et al. (2020) elucidated the difference between grasses and dicots, in perception of long and short β-1,3 glucans linked. Therefore, grasses are more sensitive to long- and short-chain glucans, whereas dicots are only sensitive to long-chain glucans. This may be related to the difference in the composition of the cell wall between grasses and dicots, where plants such as barley, rice, sorghum, and wheat are constitutively linked to β-1,3 glucans on their walls.

Given that the shiitake polysaccharide fractions exhibited biological activity, they were used in further experiments to elucidate their modes of action. In the present study, PS1 was effective in reducing the severity of *B. maydis* infection in both maize hybrids. However, it did not promote increases in the activities of defense enzymes at the evaluated time points (up to 72 h after inoculation). This could be explained by the use of a higher molecular weight polymer. The degradation of PS1 on the leaf surface generates long-term effects through the formation of oligosaccharides that can be recognized as elicitors. Like PS2, this fraction did not show direct activity against the phytopathogen in a spore germination test. However, it may have activated defense mechanisms that were not evaluated in the present study.

The potential difference in the sizes of the fractions could also explain why the PS2 fraction was effective in activating GPX for P1630H and PAL in both materials,

given that smaller fragments easily diffused through the cell wall to connect with receptors as a result of brief signaling.

The increase in PAL activity in hybrids treated with PS2, 72 h after challenge with the pathogen, may have resulted in the synthesis of antioxidant compounds and lignin precursors, such as p-coumaryl alcohol, synaphyl alcohol, and coniferyl alcohol. These precursors could contribute to the regulation of ROS to levels that do not result in HR and strengthen the cell wall (Lattanzio et al. 2006; Pandey et al. 2017).

In addition to stimulating defense enzymes in maize plants, PS2 did not inhibit the germination of the pathogen, suggesting that its mode of action occurred through the induction of resistance. This fraction, promoting disease control in only one of the hybrids, reinforces this hypothesis because the activation of plant defenses depends on the type of receptor proteins and molecules involved in intracellular signaling, which vary according to plant genotype.

Conclusion

Acibenzolar-S-methyl and aloe polysaccharides are not alternatives for the control of Southern Leaf Blight (*B. maydis*), while shiitake polysaccharides could have induced resistance against the pathogen, especially the PS2 fraction, which did not show antimicrobial activity and promoted the activation of defense enzymes in maize.

Statements and Declarations

Conflict of interest. The authors have no conflicts of interest to declare.

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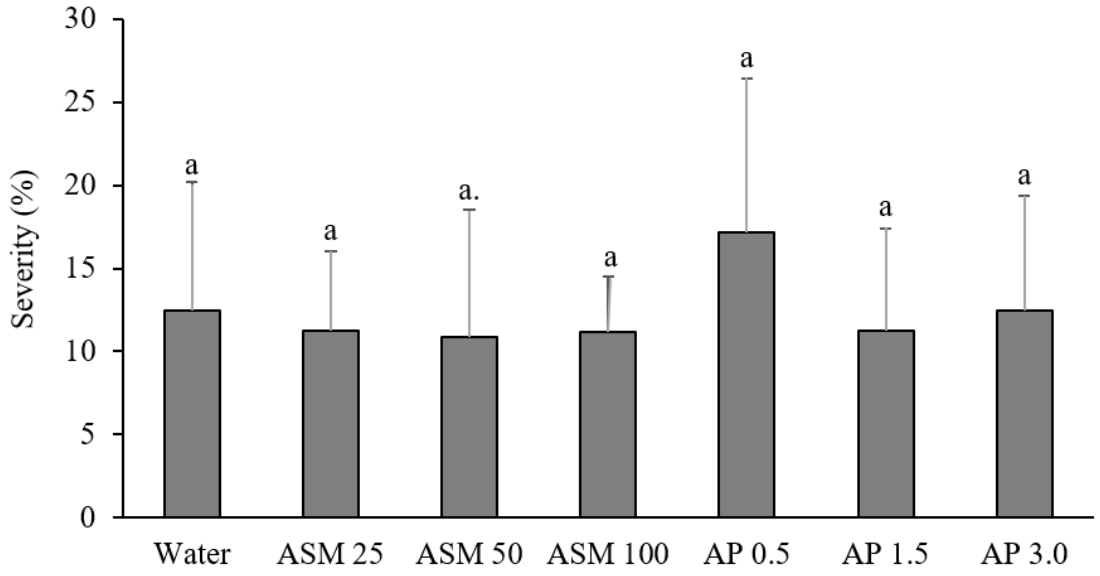

Figure 1. Severity of Southern Leaf Blight in maize plants (P1630H) sprayed with distilled water, acibenzolar-s-methyl (ASM) at 25, 50 and 100 mg.L^{-1}, or aloe polysaccharide (AP) at 0.5; 1.5 and 3.0 mg.mL^{-1} 5 days before inoculation with *Bipolaris maydis* (1×10^{3} conidia.mL^{-1}). Severity assessment was performed at 10 days after the inoculation (DAI). Means followed by the same letter present no significant difference within the same moment of evaluation, by the Tukey's test at 0.05. The vertical bars represent the standard deviation of the means.

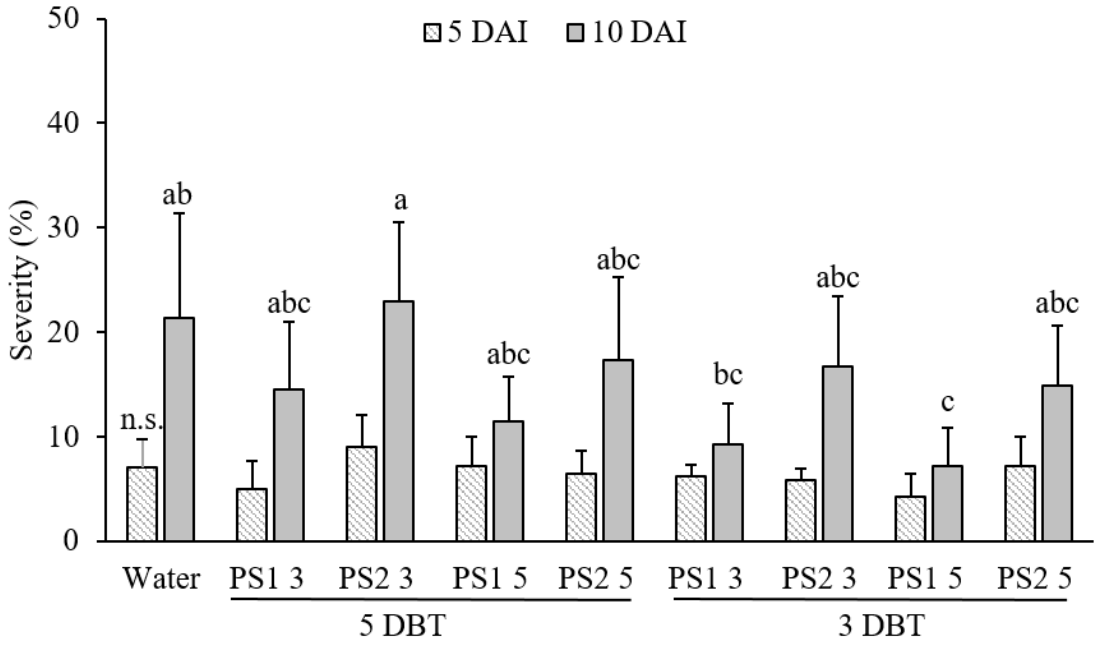

Figure 2. Severity of Southern Leaf Blight in maize plants (P1630H) sprayed with distilled water or with shiitake polysaccharide fractions (PS1 and PS2) at 3.0 and 5.0 mg.mL^{-1}, in time intervals of 3 or 5 days before inoculation (DBT) with *Bipolaris maydis* (1×10^3 conidia.mL^{-1}). Severity assessment was performed at 5 and 10 days after inoculation (DAI). Means followed by the same letter present no significant difference within the same moment of evaluation, by the Tukey's test at 0.05. n.s: not significant. The vertical bars represent the standard deviation of the means.

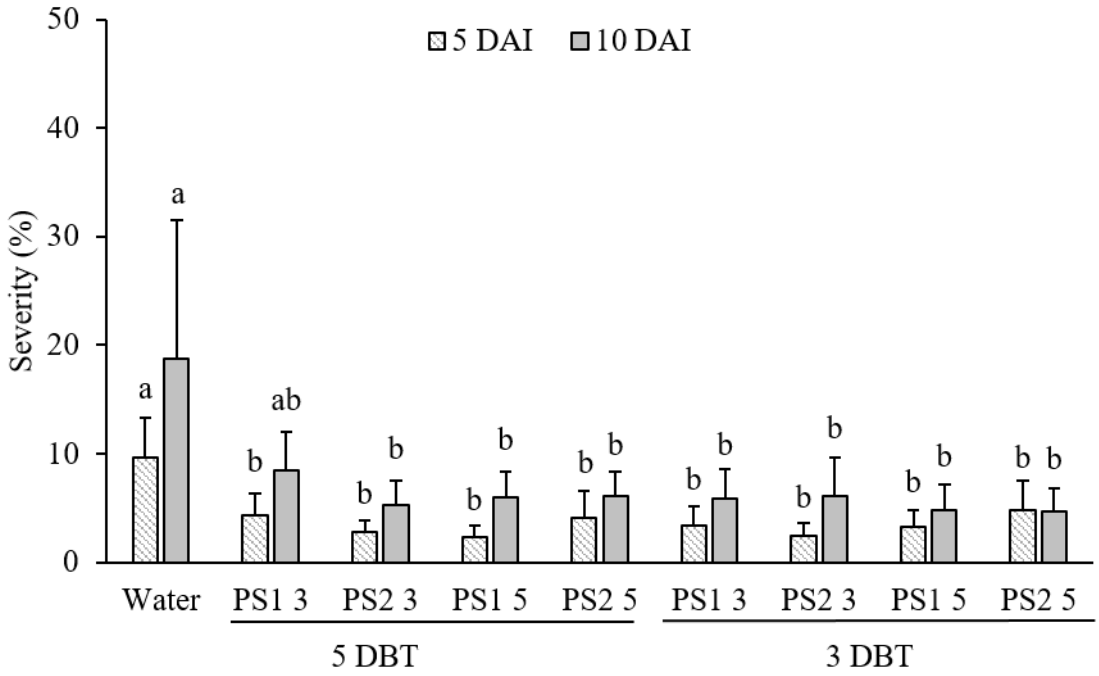

Figure 3. Severity of Southern Leaf Blight in maize plants (BM3063) sprayed with distilled water or with shiitake polysaccharide fractions (PS1 and PS2) at 3.0 and 5.0 mg.mL^{-1}, in time intervals of 3 or 5 days before inoculation (DBT) with *Bipolaris maydis* (1×10^3 conidia · mL^{-1}). Severity assessment was performed at 5 and 10 days after inoculation (DAI). Means followed by the same letter present no significant difference within the same moment of evaluation, by the Tukey test at 0.05. The vertical bars represent the standard deviation of the means.

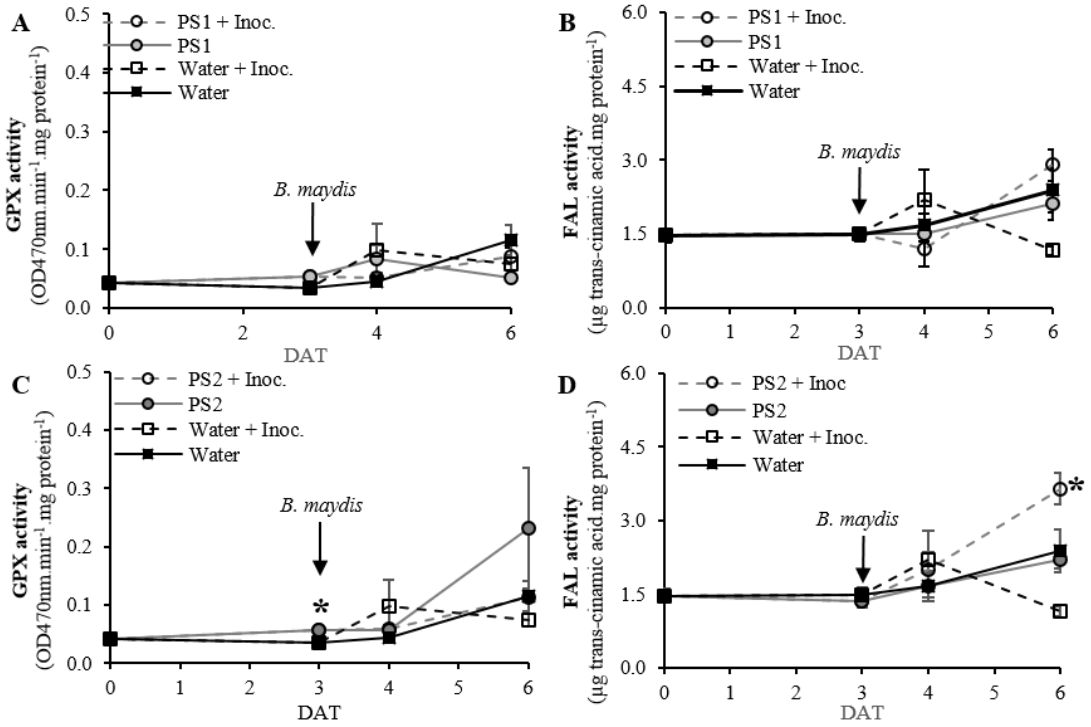

Figure 4. Activity of guaiacol peroxidase (A and C) and phenylalanine ammonia lyase (B and D) in maize plants (P1630H) sprayed with destilled water or shiitake polysaccharide fractions (PS1 and PS2) at 3 mg.mL^{-1}. The arrows indicate the moment of inoculation. PS1 + Inoc.; PS2 + Inoc.; Water + Inoc. = Plants sprayed with shiitake polysaccharide - fraction 1, - fraction 2 from shiitake or distilled water, respectively, and inoculated with *B. maydis* 3 days after; PS1, PS2 and Water = Plants sprayed with shiitake polysaccharide - fraction 1, fraction 2 or distilled water, respectively, not inoculated. Means followed by asterisks (*) show a significant difference in relation to the non-inoculated control at 3 DAT (Figure C), and to the control inoculated at 6 DAT (Figure D) by the Dunnett's test at the significance level of 0.05. The vertical bars represent the standard errors of the means.

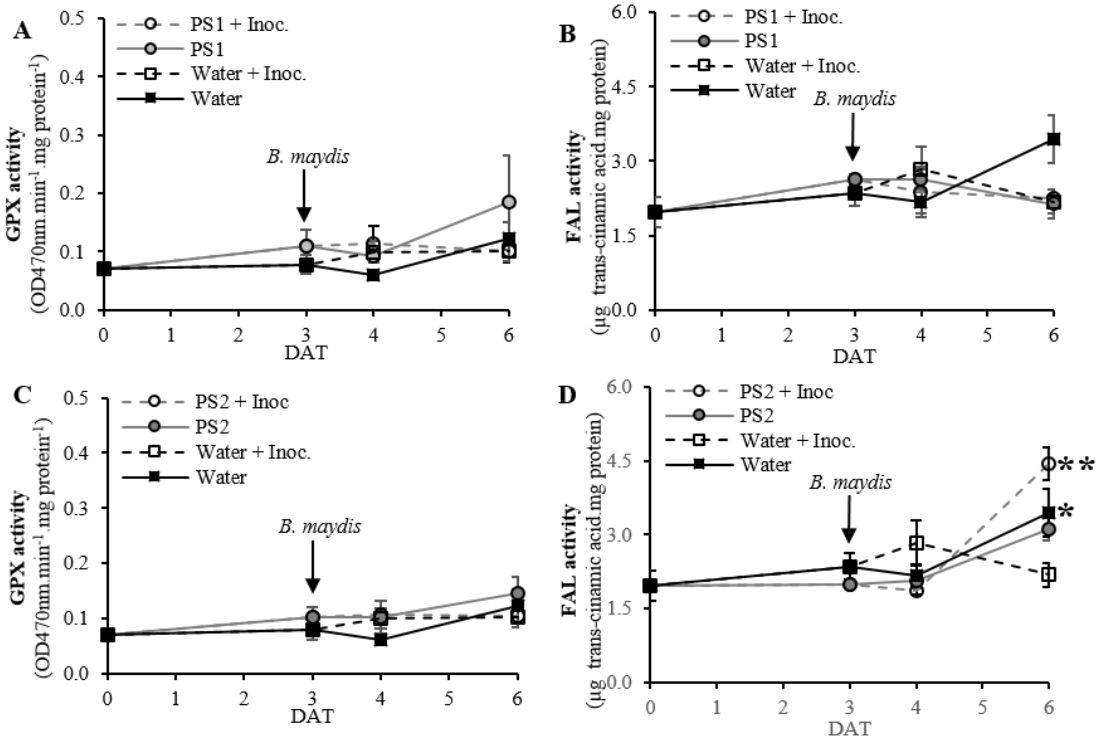

Figure 5. Activity of guaiacol peroxidase (A and C) and phenylalanine ammonia lyase (B and D) in maize plants (BM3063) sprayed with destilled water or shiitake polysaccharide fractions (PS1 and PS2) at 3 mg.mL^{-1}. The arrows indicate the moment of inoculation. PS1 + Inoc.; PS2 + Inoc.; Water + Inoc. = Plants sprayed with shiitake polysaccharide - fraction 1, - fraction 2 from shiitake or distilled water, respectively, and inoculated with *B. maydis* 3 days after; PS1, PS2 and Water = Plants sprayed with shiitake polysaccharide - fraction 1, fraction 2 or distilled water, respectively, not inoculated. Means followed by one or two asterisks (*) show a significant difference in relation to the control inoculated (Figure D), by the Dunnett's test at the significance levels 0.05 and 0.001 respectively. The vertical bars represent the standard errors of the means.

Table 1. Spore germination of *B. maydis* on maize leaves. Plants were sprayed with destilled water or shiitake polissacharides (fractions PS1 and PS2) at 3 $mg.mL^{-1}$, and inoculated with the fungus (1×10^4 spores.mL^{-1}) 3 days after. The samples were evaluated 24 h after the inoculation.

	Germination (germinated spores.cm^{-2})		
	P1630H	BM3063	Mean
Water	11.5 ± 1.6 a	11.4 ± 3.2 a	11.4 ± 2.7 A
PS1	8.3 ± 2.8 a	15.1 ± 2.5 a	11.7 ± 3.1 A
PS2	11.4 ± 4.7 a	13.1 ± 2.0 a	12.3 ± 3.4 A
Mean	10.4 ± 2.9 A	13.2 ± 2.7 A	11.8 ± 2.9

Means ± standard deviation followed by the same letter do not differ between them by Tukey's teste at a significance level of 0.05.

Figure 1

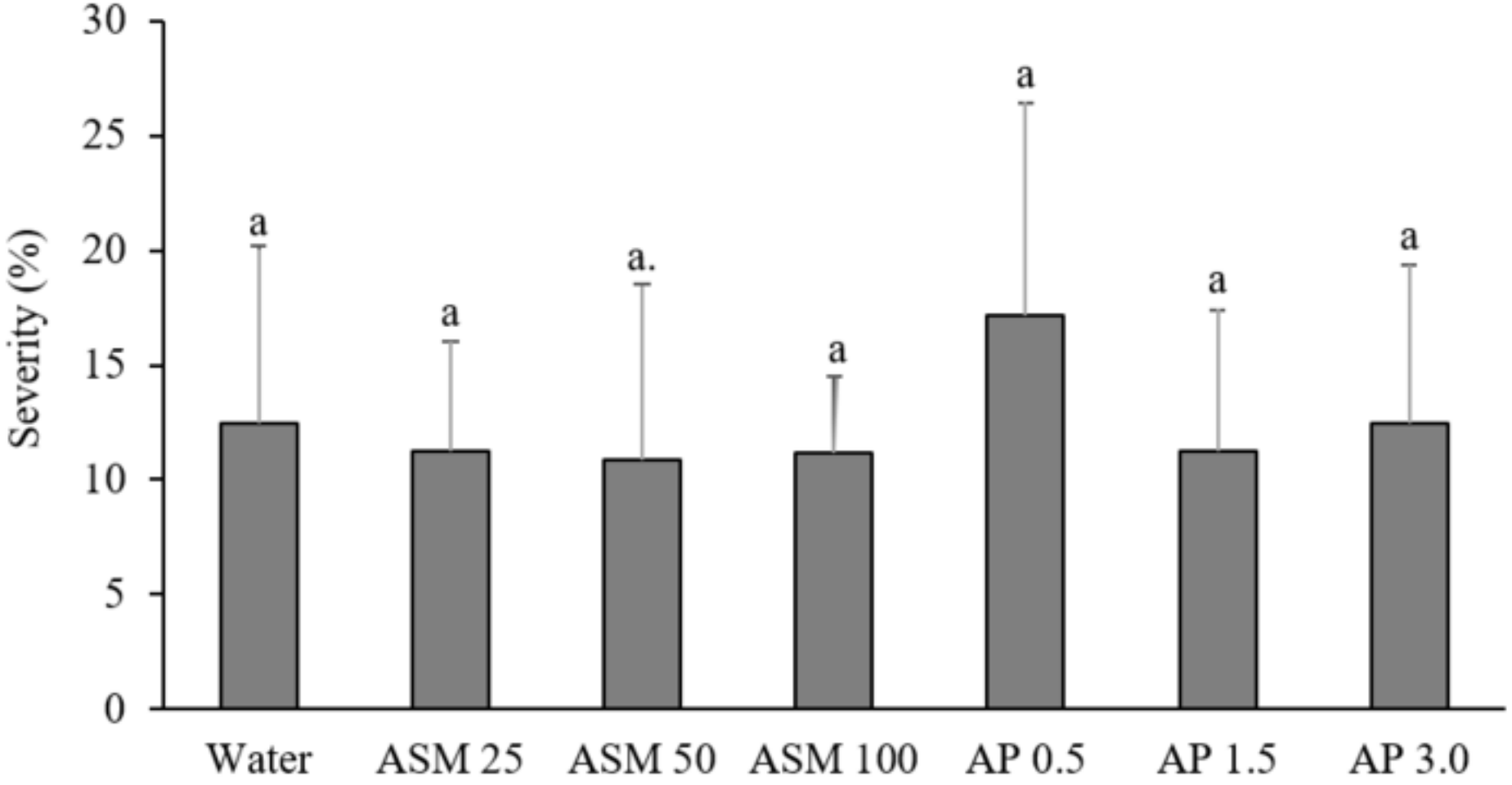

Figure 2

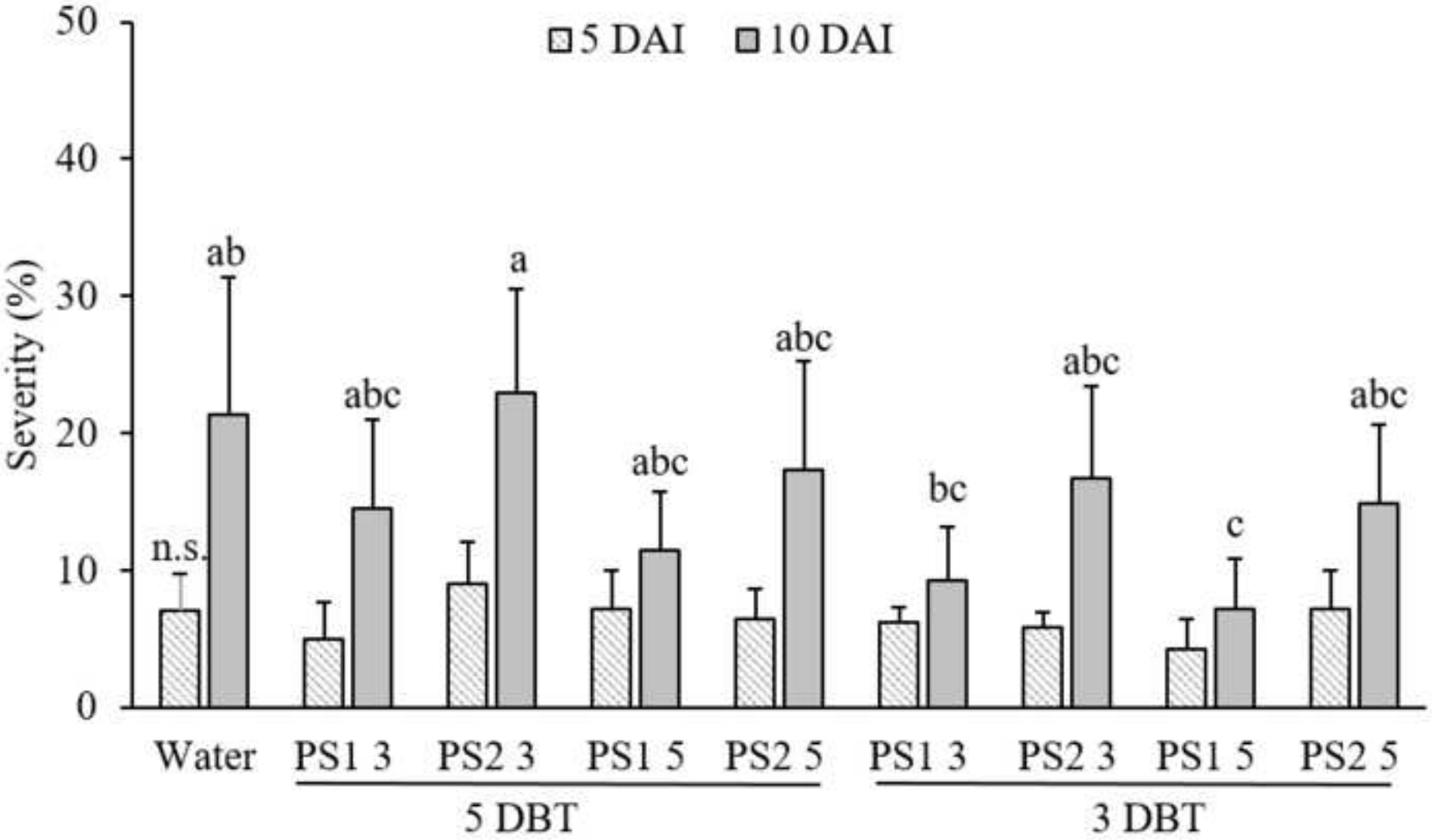

Figure 3

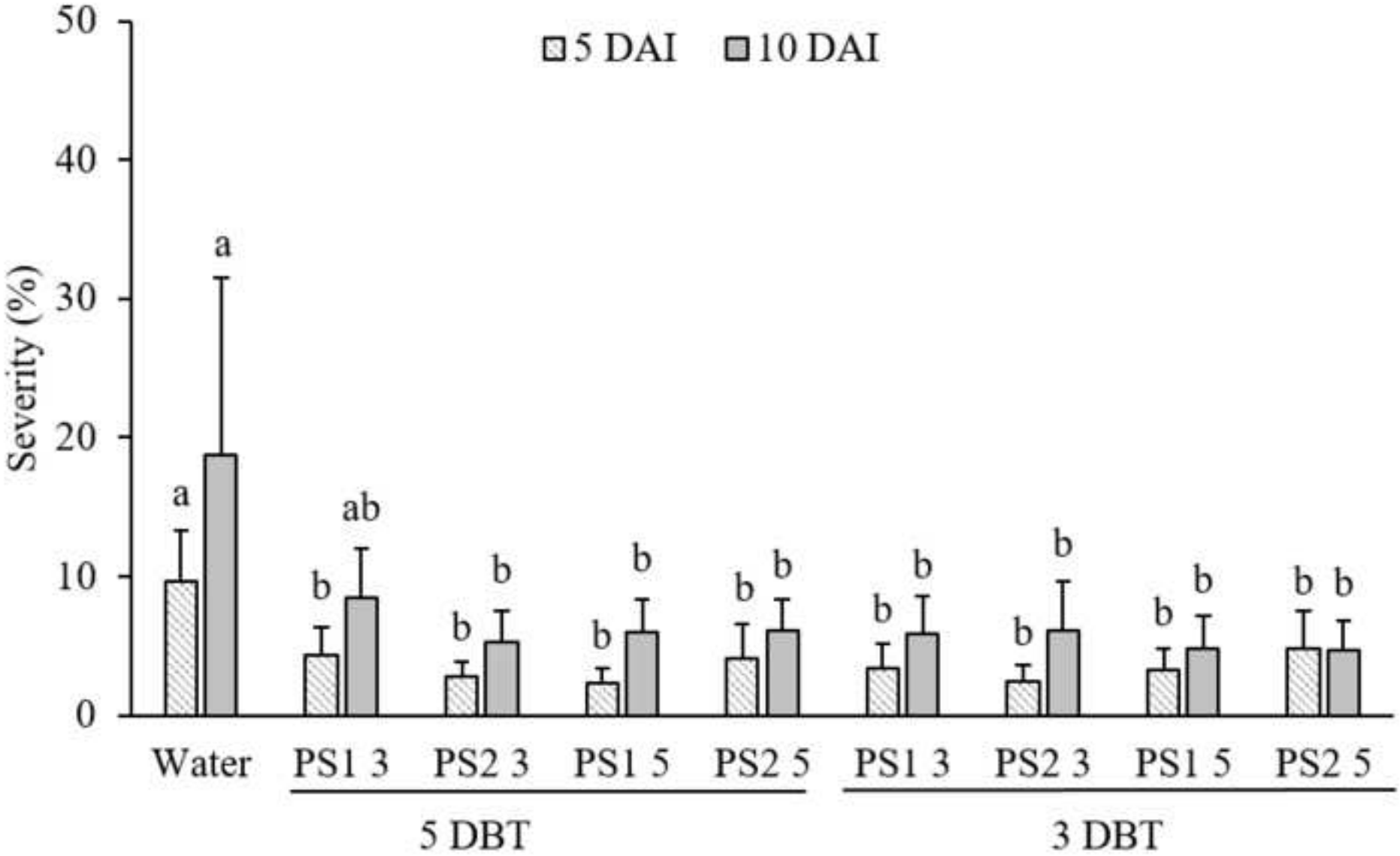

Figure 4

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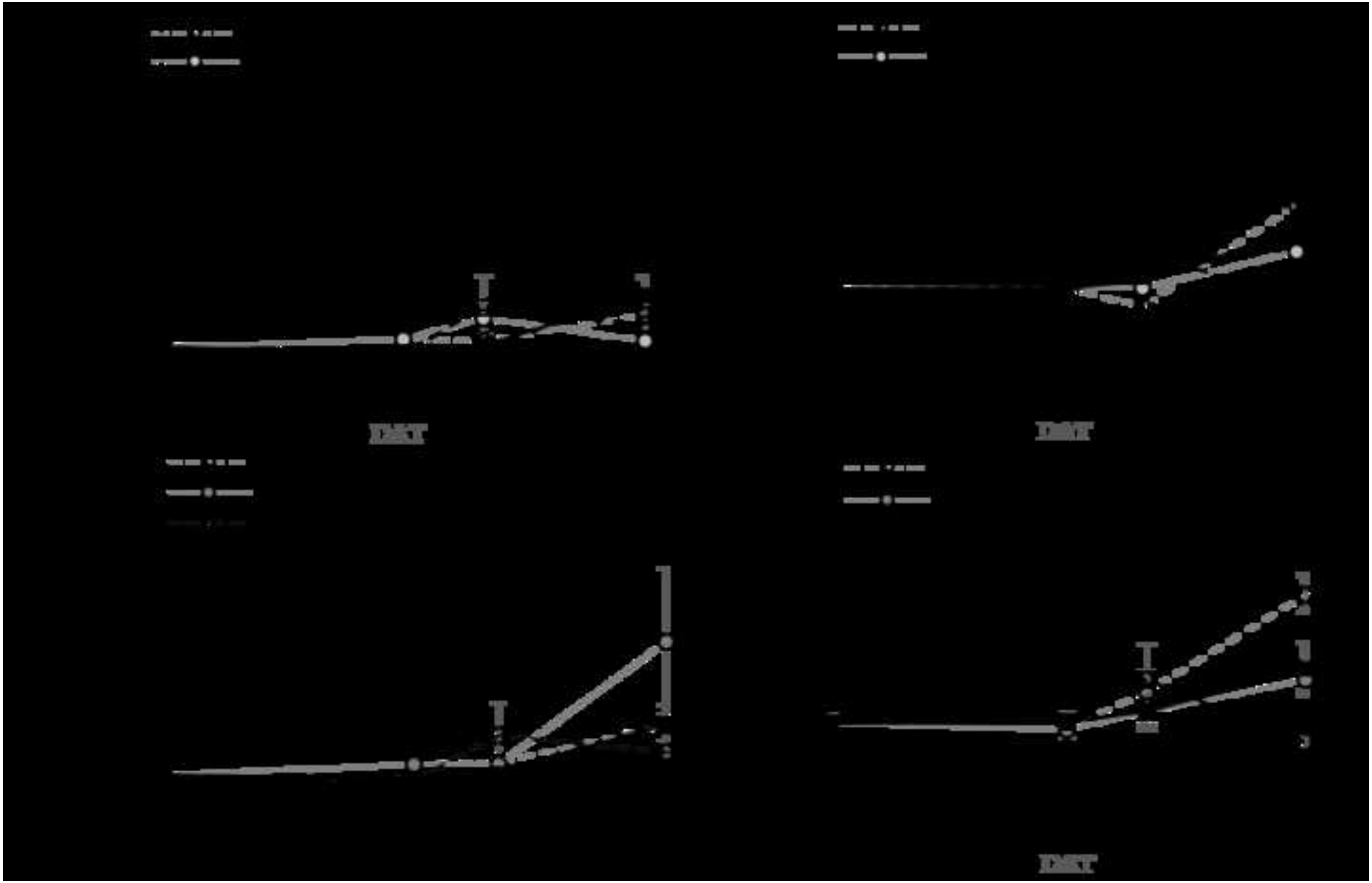

Figure 5

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