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EFFICACY OF ALLICIN (*ALLIUM SATIVUM* LINN.) AGAINST *BIPOLARIS SOROKINIANA* IN BARLEY PLANTS

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ABSTRACT

Spot blotch is caused by Bipolaris sorokiniana, and is the most deleterious disease for the producers. To control the disease, fungicides have been used that can impact the environment and human health. One method to eliminate these drawbacks is promoting induced protection. This study investigated the use of aqueous allicin extract as a biological control of B.sorokiniana or as an inducer of protection in barley plants (Embrapa BRS 195) against the pathogen under greenhouse conditions and also evaluated the possible mechanisms. Results demonstrated that induction was shown to have local and systemic action but don't have biological control in concentration of 0.097 to 0.97 µg /mL of allicin. In order to prove the resistance effect, biochemical analyses were performed to quantify proteins, phenols and the enzymatic activity of beta-glucanase. Barley plants when treated with aqueous allicin extract, showed an increased in the concentration of proteins, as well in activity of the enzyme beta-glucanase, when compared with the extract from healthy plants. In infected plants, protein concentrations decreased and enzymatic activity was lower than in healthy plants. Biochemical analyses indicated that p-coumaric acid, benzoic acid, caffeic acid and salicylic acid increased in treated barley plants. In conclusion, allicin can act as a potential elicitor that can be used as an alternative for diseases control. It's less dependent on chemical compounds, with a lower cost and causing less damage to the environment. The acting mechanism depends on the increase of salicylic acid and presence of other molecules (glucanase, proteins).

Keywords: Induced of resistance, Allicin, Barley plants, *Bipolaris sorokiniana*, salycilic acid.

Contribution/ Originality

This work is original and will contribute farmers that can be used allicin as potencial elicitor to protect barley plants against fungal disease with low cost, prevent pollution in environment and can maintenance of human health. The mechanism of action in barley plants depends of increased salicylic acid.

1. INTRODUCTION

In barley plants, several diseases caused by fungi have been detected. Spot blotch is caused by *Bipolaris sorokiniana*, and is the most deleterious disease for the producers and the beer industry. Several measures have been recommended in the control of these diseases [1, 2]. The most common one is fungicide treatment but this cause risks to the environment and to human health. Products that would induce host resistance may be a complementary management to that reduces fungicide. Induced resistance has been observed in several plants as the response to a previous treatment of the host with biotic or abiotic agents, called elicitors or inducers [3, 4]. During the host-pathogen interaction, many biochemical changes, specially the activity of beta-glucanase, proteins and phenols concentration are known to have a direct bearing with the mechanism of host resistance [3, 5]. Allicin is a major component of thiosulfinates compounds present in garlic that was produced during the process of crushing. These occur by interaction of alliin (L-(+)-S-allylcystein sulfoxide) with alliinase [6]. Cavallito and Bailey [7] found that allicin exhibit antibacterial activity against a wide range of Gram-negative and Gram-positive bacteria. That is due to its chemical reaction with thiol groups of various enzymes, e.g. alcohol dehydrogenase, thioredoxin reductase, and RNA polymerase, which can affect essential metabolism of cysteine proteinase activity involved in the virulence of bacterial [8, 9].

Miron, et al. [10] observed that phospholipid bilayers don`t constitute a barrier for allicin penetration, and that they are not disrupted.Portz, et al. [11] observed that allicin have antimicrobial action in tomato plants against *Phytophthora infestans* with reduction of disease about 45 to 100%. Other compounds as ajoene, thiosulphinates and organosulfur can be presented biocide action [12]. In addition it is conceivable that garlic extract might contain substances which are able to induce systemic acquired resistance (SAR) in the host [13, 14]. The present study investigated the use under greenhouse conditions of an aqueous extract of allicin as a biological control of *Bipolaris sorokiniana*, as an inducer of protection in barley plants against the pathogen and the possible mechanism of resistance.

2. MATERIALS AND METHODS

2.1. Extraction, Identification and Quantification of Allicin

Allium sativum cloves (1Kg) were triturated in the presence of water and ethanol (150mL water:1L ethanol), stirring in shaker for 15 minutes and incubated for seven days at 4ºC. After this time, the solution was called aqueous-alcohol extract and then filtered. The filtrate was evaporated under vacuum (at a temperature of 45ºC) for removed ethanol and remaining aqueous allicin extract (AllicinW) maintained in freezer at 4ºC [15]. One milliliter from AllicinW was

mixture with 1mL of standard solution for HPLC, more 4mL of methanol and pure water (50:50-mobile phase). The solution was filtered in a small bottle using a syringe filter (Millipore) with pore size of 0.20 µm and than maintained in freezer at 4ºC until used in high-performance liquid chromatography (HPLC). For identification molecule of allicin were used method described by Egen-Schwind, et al. [16] and Farmacopeia Brasileira [17]. The HPLC method was carried out using the GBC-HPLC instrumentation (from GBC Scientific Equipment) and analytical grade reagents from Merck-Germany. Analytical process was realized using a HPLC system with the configuration: LC1150 Quaternary Gradient Pump with 0-9,99mL/min. and pressure range 0–40 MPa, LC1445 System Organizer with manual injector Rheodine 7725 and injection volume 100µL (loop 20 µL), Flow Rate: 1ml/min, mobile phase: Methanol:water (50:50); Temperature: 27°C, Detection: UV at 254 nm, column C18 RP-HPLC**,** Silica 5 µ, pre-column C18; time for analysis: 25min and for clean 40 min; Standard: 500 mg of ethil p-hidroxibenzoate (PI) was mixture in 20mL of methanol and 900mL of pure water and put in temperature of 80ºC. The final concentration of PI solution was 0.5042mg/mL and maintained at 4ºC until analysis. The standard compound (PI) was used in chromatography for marked the final retention time because when used allicin as standard have problems because compounds are instable and can be also evaporated.

For allicin quantification was used method described by Lawson, et al. [18] in spectrophotometer that was based on Miron, et al. [19]. The better concentration was about 10^{-6} e 10^{-5}M with reaction of NTB (5,5´-dithiobis-2-nitrobenzoic acid- Sigma Chemical Co) and lecture in 412nm at spectrophotometer Shimadzu. Allicin concentration was determined in 60 minutes at the absorbance previously referred to the formula A412 x 162 (allicin molecular mass)/28300 (ε allicin), and it was equal to 1.2 µg/mL.

2.2. Source of Fungal Pathogen and Preparation of Inoculums

Isolate of *Bipolaris sorokiniana* came from infected barley leaves (Agropecuaria Foundation Guarapuava, Entre Rios, Paraná, Brazil), "cultivar Embrapa BRS 195" and kept on potato-dextrose-agar (PDA) plates. After 10 days, conidia were removed by brushing the surface of the agar and material was suspended in 10mL of sterile water followed by filtration through gauze. Concentration from pathogen was adjusted to 2 x 10^5 conidia/ mL and added Tween 20 to a final concentration of 0.05%.

2.3. Assay of Antifungal Activity

The antifungal activity of AllicinW was determined in vitroby the agar-dilution assay and observed mycelia growth and conidial sporulation. For test, one milliliter of AllicinW, in five dilutions (1:5, 1:10, 1:50, 1:100, 1:500), were incorporated in 5mL of culture medium PDA (Potato-dextrose-agar) submitted before to autoclave and after transferred in a slide of microscope and inoculated conidia of fungi (*B.sorokiniana*) and maintained in Petri plates with humidity and temperature at 27ºC. After 5 days, the area (cm^2) occupied by the fungus was

measured and conidia removed using 4mL of sterile distilled water and counted using a haemotocytometer. Three replicates were made for each treatment.

2.4. Preparation of Barley Plants and Treatment

Barley plants (cultivar Embrapa BRS 195 – from Foundation Agraria, state of Paraná, Brazil), were grown in clay pots (15cm diameter, ten seeds) containing red soil fertilized with 10N:10P:10K and micronutrients in a greenhouse under a 12h photoperiod (approximately 190 IE/m²/s) for 3 weeks when plants reached the tillering stage (stage 5) [20]. Groups of ten plants were used in each treatment and for two concentrations of allicin that didn't have inhibition in fungi. Each treatment was replicated three times and groups of plants were submitted to both local and systemic resistance induction assays. Plants were arranged in a complete randomized block design and data were submitted to variance analysis.

2.5. Induction of Local and Systemic Resistance

Protection was induced by spraying the leaves undersurfaces with AllicinW, using nitrogen gas as the propellant (0.20bars). Treatments were: (a) healthy plants (plants sprayed with water); (b) allicin (plants sprayed with AllicinW in the concentration of 0.97µg/mL or 0.48µg/mL); (c) pathogen (plants sprayed with conidial suspension); (d) plants sprayed with AllicinW (concentration of 0.97µg/mL or 0.48µg/mL) and challenge after 24 hours with conidial suspension of pathogen; (e) equal group d but challenge after 48 hours; (f) equal group d but challenge after 72 hours. After inoculation, plants were kept for 24h in a dew chamber at 100% RH, at room temperature and then maintained in a greenhouse for seven days. Percentage of protection (%P) was calculated according to Silva [21].

For systemic, AllicinW (concentration equal to 0.97µg/mL) were applied to the first up-leaf and after 24, 48, 72hours, leaves were rinsed with tap water, followed by distilled water and dried at room temperature for 2 h. After that, the first and second leaves were inoculated with the challenger. The same was made for the second leaves that was applied inducer and than in the first and second leaves was treated with conidial suspension. Plants were kept for 24h in a dew chamber at 100%RH and then maintained in a greenhouse for 4 days. Percentage of protection (%P) was calculated according to Silva [21].

2.6. Extraction and Analysis of Barley Plants Treated

Leaves from all barley plants treatments were collected and submitted to extraction. One gram of leaf material was grinding with 1mL of cold phosphate buffer (pH-7, 0.05mol/L) and incubated one hour at 4° C. After time was filtered through gauze and submitted to quantification of proteins [22] and phenols [23].

Also, 10 µL of all samples were pipette onto a thin layer chromatography plate (TLC Merck silica gel 60 F254 plates) and developed with butanol-acetic acid-water (BAW 4:1:5- organic phase). Spots were visualized at 254nm UV light and ferric chlorite (1% in alcohol). Biorad software was used to determine the area of each spot on the plate. Each front relative (Rf) value

was compared to a curve prepared by using the standard benzoic acid, salicylic acid, p-coumaric acid, quercetin, Kaempferol, ferulic acid, chlorogenic acid, cafeic acid and rutin.

For measure the activity of enzyme β-1, 3-glucanase, one gram of leaf were grinding with 10mL of cold sodium acetate buffer (0.05M pH 5.0) and incubated for one hour at 4° C. After, the crude extract was filtered through a nylon cloud to remove cell debris. The activity of enzyme was determined by measuring the rate of sugar reduction using laminarin as the substrate, dinitrosalicylic acid as reagent and, glucose as the standard, according to the methods of Van Hoof, et al. [24] and Wyatt, et al. [25].

2.7. Identification of Salicylic Acid by HPLC

Leaves from treatment A-72h (that occur induced of protection against fungi *B.sorokiniana*), control-allicin, infected and healthy plants were used for identification through HPLC. For this, one gram of barley leaves was ground in a blender with 3mL of cold sodium acetate buffer 0.05M, pH 5.0. The homogenate was incubated in a refrigerator for one hour prior to being through gauze and a 0.45µm Millipore filter. The filtered solution was treated with 0.01g of PVP (insoluble polyvinylpyrrolidone) and maintained over-night at 4°C. The precipitate was washing with 1mL of acetate buffer and after, soluble with 1mL of a methanol solution (90% methanol and 1% NH_4OH 0.01mol/L). The solution was evaporated in desiccator connected to vacuum pump and maintained for 2 days in refrigerator.

Residue was removed with 1mL of methanol:water:acetic acid (70:30:4) (Application note 43 Supelco). HPLC instrument was used from GBC-HPLC and analytical grade reagents from Merck-Germany. Analytical process was realized with the configuration: LC1150 Quaternary Gradient Pump with 0-9,99mL/min, pressure range 0 – 20 MPa, LC1445 System Organiser with manual injector Rheodine 7725, and injection volume 10µL (loop 2 µL), Flow Rate: 0.5ml/min, Temperature: 27°C, Detection: UV at 270 nm, column Supelcosil LC-18, pre-column C18; time for analysis: 25min and for clean 40 min. Mobile phase was methanol:water:acetic acid (40:60:1). In the Supelco application, salicylic acid has peak with retention time as 12 min. Results was submitted to analysis and relative peak of the sample was expressed as percentage of the peak of salicylic acid (standard).

2.8. Statistical Analysis

All experiments were performed in duplicate and analyzed by the Student's paired t-test or Origin (ANOVA) software, and the significant difference was set at $P \leq 0.05$.

3. RESULTS AND DISCUSSION

In freshly crushed garlic (*Allium sativum*) was present phenolic compounds such as flavonoids (with high antioxidant), quercetin (flavonol) [26], apigenin (flavona) and miricetin (flavonol) [16, 27]. Also in one gram of garlic have 33 compounds contained sulfur and can be found from 11 to 35mg of these compounds [28]. From sulfur active compounds the most studied was allicin and that have propriety as antioxidant [29] and have the most medicinal effects [30].

The intact garlic clove does not contain allicin but rather its precursor, the non-protein amino acid alliin. Alliin is the precursor of allicin formed by the action of allinase enzyme, when the bulb is cut or crushed [31]. The amount of allicin in fresh garlic is highly variable [30].

Allicin is oil liquid, water soluble at 2.5% with a characteristic odor (not as pungent as diallyl disulphide) [31]. Block, et al. [32] said that allicin is highly volatile but produce an instable compound and at room temperature it decomposes almost totally in a 24h period.

In order to produce a stable agent that can be used in formulations, an AllicinW (aqueous allicin extract) that stabilizes the molecule [33]. Cutler and Wilson [34] worked with aqueous Allicin extract and observed that was highly active against clinical isolates of multiple antibiotic resistant to *Staphylococcus aureus*. So, in this work was used the aqueous allicin extract (AllicinW). In the first stage of this work, allicin from freshly crushed garlic was extracted and the correspondent identification and quantification was executed. The second stage studied effect on conidia from *B. sorokiniana* and the third stage observed the effect as inducer of protection and the possible mechanism action in barley plants.

3.1. Extraction, Identification and Quantification of Allicin

The allicin after extraction from freshly garlic [15] was maintained stable in freeze at –20°C until five months. HPLC analysis revealed the following: Allicin with retention time of 7min and standard (PI) with retention time 21min. In 1.5 to 2 min was observed a peak that can be a trace from impurity (Figure 1). The results obtained were correlated with Lawson, et al. [18] that observed the same retention time for allicin. Allicin extract was stored in freezer at –20C and determined concentration about 48.5 µg /mL of allicin and the results was reproducible in five months.

3.2. Assay of Antifungal Activity

The antimicrobial action depends on allicin and is thought to be due to inhibitory effects on various thiol-dependent enzymatic systems. The antifungal activity of garlic extracts has been observed *in vitro* against *Cryptococcus neoformans*, *Candida* and *Aspergillus* spp [35, 36]. Cavallito and Bailey [7] observed that allicin has a potential to inhibit the common genera of plant pathogenic bacteria and the fungi *Alternaria brassisicola*, *Botrytis cinerea*, *Plectosphaerella cucumerina*, *Magnaporthe grisea*, and the oomycete *Phytophthora infestans*. Miron, et al. [19] explained that the mode of action of allicin in fungi or bacteria was the high intracellular reactivity with thiols groups resulting from high membrane permeability.

All research with plant pathogenic fungi was with biotrophic fungi. The present work used a necrotrophic fungi and observed that had another action because don't presented high inhibition with allicin. The results obtained in development of *B.sorokiniana* above medium with AllicinW, showed that extract didn't demonstrate difference in growth and production of conidia when compared to the control slide, in three dilutions (0.97, 0.48 and 0.097 µg /mL) of allicin because didn`t present a antimicrobial control (Table 1). When AllicinW is incorporated in agar in other

concentrations about 4.85 and 9.7 µg /mL of allicin, occurred inhibition in growth and production of conidia from 5 to 10% (respectively) was obtained (Table 1).

3.3.Induction of Local and Systemic Resistance

Cavallito and Bailey [7] explained that garlic extract with water might contain substances which are able to induce systemic acquired resistance (SAR) in the host. In results from present work allicin didn't present biological control above conidia from *B.sorokiniana*, in three concentrations of allicin about 0.97; 0.48 and 0.097µg /mL. But local effect of induction against *B.sorokiniana* in barley plants was observed by means spraying AllicinW in the whole plant (Table 2). According to results, two different concentrations of allicin were effective in inducing protection against fungiin barley plants. With 0.48µg/mL of allicin the level of protection was between 79 at 100% and with 0.97µg/mL of allicin, the level was between 80 to 100%. The protection was increased with the time from 24 to 72h when compared with infected plants (Table 2). Similar results were obtained by Naganawa, et al. [37], who also observed in barley plants better protection in the 72 hours interval between challenge (xanthan gum) and *B.sorokiniana*.

Data was not represented but when used the concentration of 9.7µg/mL of allicin for pulverizing the barley plants, some leaves presented aspect of burnet.

For observed systemic protection was used the concentration about 0.97 µg/mL of allicin that was corresponded a better protection. The leaf in plant was demarked as: Leaf 1 is the first above (new leaf); Leaf 2 the second leaf is the lower (oldest leaf) in the plant. The allicin was applied 24h, 48 and 72h before challenge inoculation. It is interesting to note in results that cultivar demonstrated 100% of protection, with both a ascending and descending systemic effect in the interval of time of 48 and 72h between challenge and pathogen. However when plants were submitted to treatment in 24h of interval, the descending systemic effect was higher as to ascending effect (Table 2). These data are in accordance with those by Kuc [38], Bach et al.[3], in other plant–pathogen systems. The system was important because can sprayed the plant at the lowest leaf or in other local of the plant but the signal occur and translate to all leaves.

3.4. Analysis from Treated and Untreated Barley Plants

Treated plants with AllicinW presented protein and phenols at the same quantity of control plants (healthy or control with allicin). In time of A-72 hours the protection was higher and presented more proteins and decreasing phenols. Infected plants presented more phenols and decrease proteins (Table 3). Similar results were obtained by Bach et al. [3, 39], Castro and Bach [5], who also observed in barley plants better protection and more proteins and decreased of phenols.

Various authors said that the response of plant to pathogen includes alterations of the cell wall, production of phenolic metabolites and reactive oxygen intermediates, a hypersensitive cell death reaction (HR) as well as accumulation of pathogenesis-related (PR) proteins [12, 40, 41]. In some systems, the expression of selected PR-proteins, e.g. beta-1,3 glucanase, upon inducer treatment has been used as an indicator for induced resistance. Results demonstrated that β-1,3

glucanase activities was present in locally and systemically treatments with AllicinW. Control plants (sprayed with water) presented enzymatic activity as 6.7 and control (sprayed with AllicinW) presented activity as 4.2. In infected plants the activity was decreased as 2.3 when compared with control plants. Enzyme activities in the locally protected leaves in interval of 72h using elicitor with 0.97μg/mL of allicin, demonstrating 100% of protection with enzyme activity equal to 9.3 (Table 3). Systemic protection may be correlated with the increase in the enzymatic activity of β-1,3-glucanase mainly when leaf 1 was treated and leaf 2 of the same plant demonstrated to have received some kind of signal from the other leaf, with prevented the infection from the pathogen, even when it did not receive the inducer. In the systemic effect was observed that protection exist in the ascending route in time of 72hours but, in 24 and 48hours, occur in descending route and that was correlated with enzyme activity (Table 3). So, the results can be explained that the treatment with AllicinW can protect the barley plants in the two directions ascending and descending. These data are in agreement with those obtained by Bach, et al. [3], Castro and Bach [5] and by Bach, et al. [39] for barley and xanthan gum; Jenns and Kuc [41] , Zhang and Yuen [42], in other plant-pathogen systems.

3.5. Thin Layer Chromatography

Dixon [43] described that compounds from secondary metabolism was generally from complex nature and restrict distribution in plant. In plant can occur changes in metabolism that including accumulation of phytoalexins and aromatic cell wall bound compounds.

In this work, quantity of phenols decreased in plants treated with AllicinW but, in infected plants, the phenols increased. One reason for this is that fungi *B.sorokiniana* attack the cell wall in leaves from barley and growth into the cells even the cells are dead. So, *B.sorokiniana* are a necrotrophic pathogen and may be expected to cause extensive tissue damage and that could be explained the increase of phenols. Phenolic compounds came from secondary metabolism derived from aromatic amino acids phenylalanine and tyrosine and can be observed through thin layer chromatography (TLC) [44].

Standard benzoic acid in TLC didn't visualize fluorescent area with UV light but gave brown color when spraying solution of $KMNO_4$ 0.1% in water and disappear after 10 min (Rf=0.74). For cafeic acid and p-coumaric acid under UV light observed fluorescent area in RF determined as 0.84 and 0.68 respectively. In sample from allicin (A72h) that occurred higher protection against *B. sorokiniana*, presented in total six bands but only three bands could be identified as p-coumaric acid, benzoic acid and cafeic acid. In extract from control plants (treated only with allicin) was observed eight bands but three came from healthy plants and the other five are initially formed that is: Rf=0.420, 0.594, 0.683, 0.740 and 0.840. In healthy was observed five bands and in infected plants was observed only one and that could be explained that the other bands was inhibited by attack of fungi (Figure 1).

The interaction between plants and necrotizing pathogens often leads to the development of resistance to subsequent infection. This defense response is not only restricted to plant tissues in contact with the pathogen. Pathogen-free parts of the inoculated plant also become resistant. This

phenomenon called systemic acquired resistance (SAR) [38]. The development of SAR is usually preceded by a hypersensitive response characterized by the formation of necrotic lesions around the site of infection. This primary defense response in the inoculated parts of the plant is accompanied by an array of biochemical changes. These include generation of active oxygen species, cell death, overproduction of phenolic compounds, deposition of lignin-related materials, and induction of the expression of pathogenesis-related (PR) proteins. The occurrence of SAR in response to a pathogen requires a long-distance transport of a factor originating in the tissue expressing the hypersensitive response that moves systemically to other parts of the plant. It was suggested that salicylic acid (SA) is likely to be the molecule responsible for SAR of plants to pathogens [10, 44, 45].

The biosynthesis of SA was suggest by Lee, et al. [46] that plants resistance, synthesized SA, from cinnamic acid by two possible pathways: 1) involves side-chain decarboxylation of cinnamic acid to benzoic acid followed by 2-hydroxylation to SA. 2) cinnamic acid could be first 2-hydroxylated to o-coumaric acid and then decarboxylated to SA. The results in work demonstrated that plants when treated with allicin and than spray with conidia, presented p-coumaric acid, benzoic acid and cafeic acid that can be activated the phenylpropanoid patway.

3.6. Identification of Salicylic Acid on Extract of Barley Plants

Samples from barley plants treated with allicin (A-72h), control allicin (plant in 72hours only sprayed with allicin), health plants and infected plants was after extraction filtered in Millipore, and obtained a transparent filtrate. According to Sheonjin [45] salicylic acid can be absorbed on the PVP under acidic condition and was desorbed from the PVP under alkaline condition and so, was used the method for HPLC.

Results demonstrated that salicylic acid was removed from PVP and detected in HPLC. In Table 4 observed that standard of allicin have retention time 7 min and standard salicylic acid 12min (peak height=24%). When observed healthy plants, four peaks appeared and one was related with retention time of salicylic acid with height peak (0.5%) and the other three bands cannot be related with a standard known compound.

It was interesting that in allicin control plants was observed in 4min of retention time a higher peak (32%) than compared with healthy plants (4.2%). In 14 and 24min of retention time the results were identical. When sprayed allicin in plant a reaction in cell wall occur and a compound can be increased at 4 min (32%) but when the plant was treated with allicin and fungi, the peak decreased or can be inhibited by attack of fungi because denoted peak height at 12.4%. In control plant (only with allicin) was observed 5% of allicin in leaves (Table 4).

In plants from group A-72h the retention time of salicylic acid presented one higher peak (19.2%) when compared with control allicin plant (0.9%) and health plant (0.5%). So, barley plant when treated with allicin and after 72hours have attack of fungi, present 10% more salicylic acid.

The nature of the mechanisms responsible for the induction of resistance caused by allicin in barley plants denoted production of salicylic acid and also presence from p-coumaric acid, benzoic acid and cafeic acid that could be responsible for biosynthesis from SA at pathway of activation of

phenylpropanoid acid. Also in results can be observed that genes of beta-1,3 glucanase was induced. In conclusion, allicin could be used as a resistance inducer in barley plants but didn't for biological control above conidia of *B.sorokiniana.*

In summary, allicin can act as potential elicitor that can be used as an alternative of disease control, less dependent on chemical compounds, with a low cost, causing less damage to the environment and the action mechanism depends of increased salicylic acid. When allicin was used in higher concentration, growth and production of *B. sorokiniana* conidia was inhibited demonstrating mechanism of biological control.

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Table-1. Development and production of conidia from *B.sorokiniana* submitted to different concentrations of allicin from extract AllicinW.

	dilution of extract = µg /mL of allicin	**Number of conidia x 10^4****	**Total area (cm)***	**% of inhibition of area****
AllicinW	1:5= 9.70	1.58b	1.8b	10
	1:10= 4.85	1.82c	1.9c	5
	1:50= 0.970	2.00a	2.0a	0
	1:100= 0.480	2.00a	2.0a	0
	1:500 = 0.097	2.00a	2.0a	0
Control (*B.sorokiniana*)	X	2.00a	2.0a	

*Media of three repetitions. Same letters in columns was not different statistically when compared with control. Different letters in columns were different statistically compared with control ($p<0.05$ teste T Student`s).

** % of inhibition compared the treatments with control slide.

Table-2. Induced of protection in barley plants cultivar Embrapa BRS-195 agaisnt *B. sorokiniana* and treated with AllicinW (A) in different concentrations and times between elicitor and pathogen.

Treatment local	Allicin (□g/mL)	% protection[1]	Treatment systemic	% protection[1]
Health	--	--	A-24h Sist1T	70 b,b
Allicin control	--	--	Sist 1 F2	90 b,c
infected	--	0.00 a	A-24h Sist 2 F1	70 b,b
A-24h	0.48	79.16 b,c	Sist 2 T	80 b,d
A-48h	0.48	85.00b,d	A-48h Sist1T	88 b,e
A-72h	0.48	100.00b,e	Sist 1 F2	88 b,e
A-24h	0.97	80.14b,f	A-48h Sist 2 F1	90 b,c
A-48h	0.97	93.60b,g	Sist 2 T	90 b,c
A-72h	0.97	100.00b,e	A-72h Sist1T	100 b,f
			Sist 1 F2	100 b,f
			A-72h Sist 2 F1	100 b,f
			Sist 2 T	100 b,f

[1]Media of three repetitions. First letter: different letter are significantly different from infected plant (P<0.05 Student's t-test and Origin-ANOVA). Second letter after coma: different letter are significantly different from other plants in group (P<0.05 Student's t-test and Origin-ANOVA) and same letter was not significantly different from other plants in group or concentration of allicin.

Fig-1. Thin layer chromatography from barley plants treated with allicin. Bars = Rf (mobility ratio) correspondent to area (mm²). Results compared with Rf from standard p-coumaric acid, benzoic acid, cafeic acid.

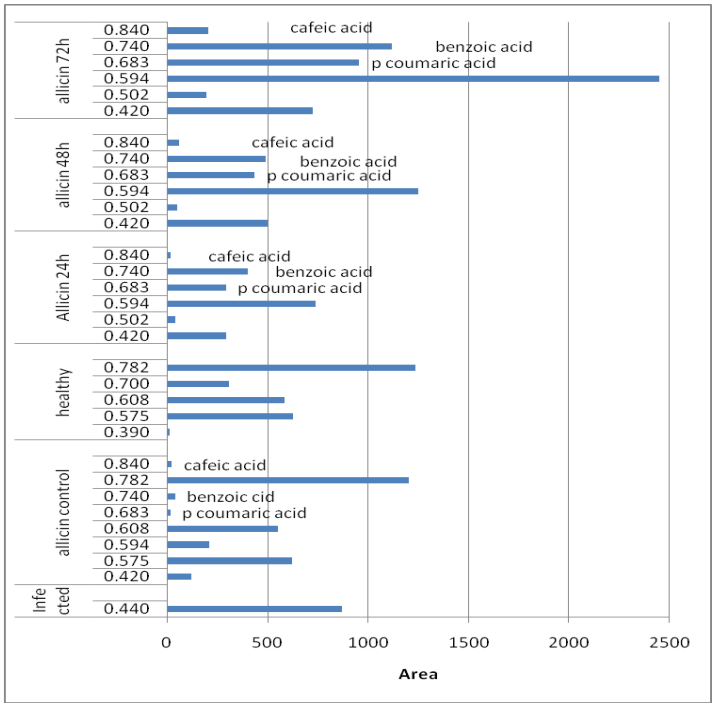

Table-3. Activity of beta 1,3 glucanase, concentration of proteins and phenols present in barley plants submitted to treatments with AllicinW (A)against *B. sorokiniana.*

Treatment local induction	**Allicin (µg/mL)**	**Activity beta-1,3 glucanase[1]**	**mg of SAB/g fresh leaves**	**mg chlorogenic acid/ g fresh leaves**
Health	--	6.7 b	12.94 b	0.72b
Allicin	--	4.2 b	10.28b	0.62b
infected	--	2.3 a	05.80a	0.94a
A-24h	0.48	6.6 b	10.65b	0.52b
A-48h	0.48	6.7 b	11.11b	0.45b
A-72h	0.48	7.1 b	13.65b	0.31b
A-24h	0.97	8.7 b	09.95b	0.59b
A-48h	0.97	8.9 b	10.00b	0.48b
A-72h	0.97	9.3 b	12.90b	0.30b
Treatment Systemic induction	Type of protection	Activity beta-1,3 glucanase[2]	mg of SAB/g fresh leaves	mg chlorogenic acid/ g fresh leaves
A-24h Sist1T		8.3a	9.05a	0.5
Sist 1 F2	Descendent	9.8b	9.90b	0.5
A-24h Sist 2 F1		7.1c	9.32c	0.5
Sist 2 T	Descendent	8.0d	9.90b	0.5
A-48h Sist1T		8.8a	9.9a	0.4
Sist 1 F2	Descendent	9.8b	10.0b	0.4
A-48h Sist 2 F1		8.8a	9.8c	0.4
Sist 2 T	Descendent	9.8b	10.0b	0.4
A-72h Sist1T		11.0a	12.2a	0.3
Sist 1 F2	Ascendant	10.8b	12.0b	0.3
A-72h Sist 2 F1		11.1c	12.0b	0.3
Sist 2 T	Ascendant	10.5d	11.9c	0.3

[1]Media of three repetitions. Same letters in columns was not significantly different from infected plants. Different letters in columns was significantly different from infected plants ($P<0.05$) Student's t-test and Origin (ANOVA)

[2]Media of three repetitions. Different letters in columns (same group) was significantly different from other group. Same letters in columns (same group) was not significantly different when compared with group plants.

Table-4. Identification of bands in HPLC-GBC in barley extracts from plants submitted to treatment with AllicinW (A) against *B. sorokiniana*.

Treatments	retention time (min)	Relative peak height (%)	Identification
Standart AllicinW	7	98	
Standart salicylic acid	12	100	
healthy	4	4.2	
	12	0.5	salicylic acid
	14	44	
	24	38	
AllicinW control	4	32	
	7	5	Allicin
	12	0.9	salicylic acid
	14	44	
	24	41	
AllicinW 72horas	4	12.4	
	12	19.2	salicylic acid
	14	49	
	24	46.4	
Infected	2	6	
	4	4.4	
	9	9.8	
	14	3.3	