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Induction of defense mechanisms from filtrates of saprophytic fungi against early blight disease in tomato

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The induction of defense enzymes presents efficient plant disease management when triggered by metabolic products of microorganisms. The aim of the present study is to select filtered saprobes fungi used for the management of early blight of tomato by inducing pathogenesis related to tomato plant. Filtrates of the fungi *Curvularia eragrostidis*, *Curvularia inaequalis, Memnoniella echinata* and *Pseudobotrytis terrestris* were cultured in potato and dextrose (PD) media and maintained in a growth chamber at 25 ± 2°C, with a photoperiod of 12 h light. After 20 days, the mycelial mass was removed through filtration. Concentrations (0, 5, 10, 15 and 20%) of filtrates and Acibenzolar-S-Methyl were applied in the 3rd tomato leaf 3 days before inoculation with *Alternaria solani*. Then the disease severity was analyzed calculating the area under the disease progress curve. The activity of the enzymes, catalase, guaiacol peroxidase and phenylalanine ammonia-lyase (locally and systemically) at plant control (72 h before inoculation) and 0, 24, 48, 72 and 96 h after inoculation with *A. solani* was analyzed. *C. eragrostidis*, *C. inaequalis*, *M. echinata* and *P. terrestris* filtrates reduced the AUDPC, both locally and systemically. Greater activity of phenylalanine ammonia-lyase was observed in plants treated with *C. eragrostidis* (local and systemic) and *C. inaequalis* filtrates (local). The filtrate of *P. terrestris* promoted greater catalase activity, either locally or systemically. The filtrate of *M. echinata* increased peroxidase activity locally and systemically. The filtrates tested are resistance inducers used for the management of tomato early blight, although further testing is necessary to identify elicitors present in filtrates.

Key words: *Alternaria solani*, alternative control, proteins related to pathogenesis, *Lycopersicon esculentum*.

INTRODUCTION

Lycopersicon esculentum is one of the most important crops in the world agricultural scenario. It constitutes an important product for "in natura" trade and extract industry. It generates direct and indirect employment. However, tomato production is considered a risky activity because the culture is affected by many diseases. Among these, tomato early blight, caused by the fungi *Alternaria solani*, is a major disease which causes great losses for producers, as it is a highly destructive disease and of rapid proliferation. It focuses on leaves, stems,

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petioles and fruits of tomato. Studies conducted by Tofoli et al. (2003) on conditions of Brazil observed that early blight, without control, can cause 57% loss in yield of commercial fruits and cause qualitative losses (55%) through burning of fruits caused by defoliation.

Crops that are highly susceptible to pathogens require the adoption of constant pesticide applications during cultivation. The awareness of the problems generated by the adoption of isolated methods for disease management by chemical methods has increased research technologies such as inducing plant resistance to pathogens.

Induction of resistance is a method that promotes the reduction of disease severity. This method consists of sensitizing the plant to activate its defense mechanisms by an elicitor agent, and preparing the plant for the pathogen arrival, its infection and colonization (Conrath et al., 2002). The activation of plant defense system involves an increase in the enzymes activity related to pathogenesis, such as peroxidase, phenylalanine ammonia-lyase and catalase (Garcia-Cristobal et al., 2015; Maschietto et al., 2016).

Among the reported elicitors, there are biotic agents such as microorganisms, which can activate plant defense mechanisms (Chowdappa et al., 2013). Resende et al. (2015), exploring the biodiversity of saprobe fungi present in semi-arid North-east of Brazil, observed that conidial suspension of *Curvularia inaequalis* was able to potentiate sorghum resistance against *Colletotrichum sublineolum* infection. Filtrates of pathogenic fungi, non-pathogenic and saprobes are able to activate the defense system because they contain molecules: proteins, oligosaccharides, oligopeptides, toxins and others. They function as microorganism recognition signature, which is perceived by protein receptors present in the cell membrane of the plant cell (Dubery et al., 2012).

In proposing research to identify inducers of plant resistance to pathogens, it is important to note the potential for inhibiting or reducing the symptoms of pathogen attack and to study the biochemical changes occurring in the host. Further, persistence of defense response(s) involved, as well as systemic signaling should also be considered (Choudhary and Johri, 2007).

This current work aimed to study the effect of different concentrations of saprophytic fungal filtrates in induction of defense related enzymes against early blight disease of tomato.

MATERIALS AND METHODS

The experiments were conducted in the Laboratory of Alternative Control and Induction of Resistance at the State University of Maringa, from January 2012 to December 2013, in the City of Maringá, State of Paraná.

Preparation of *A. solani* inoculum

The pathogen *A. solani* was obtained from the State University of Western Paraná, and plated on Potato, Dextrose, and Agar (PDA) at 25 ± 2°C for 2 to 3 days to obtain pure colonies. The pure phytopathogen was kept in a growth chamber at 25 ± 2°C in PDA, with a photoperiod of 12 h light.

Preparation of filtrates from fungal saprobes

Isolates of saprobes fungi from Brazil's semi-arid Northeast (*Memnoniella echinata*, *Curvularia eragrostidis*, *C. inaequalis* and *Pseudobotrytis terrestris*) ceded by the State University of Feira of Santana, and deposited in CCMB (Bahia Microorganisms Collection) were grown on PDA. They were kept in a growth chamber at 25 ± 2°C, with a photoperiod of 12 h light.

The filtrates were obtained by subculturing fungal mycelia disc grown on PDA to 100ml of Potato dextrose broth sterilized at 120°C and 1 atm for 20 min. They were incubated in a growth chamber at a temperature of 25 ± 2°C with a photoperiod of 12 h of light for 20 days. Later, the broth containing fungal liquid metabolites was filtered through Whatman paper No. 1.

Effect of fungal filtrate on early blight incidence and defense related enzymes

To study the influence of filtrates' concentrations and induced biochemical aspects, tomato seeds from the cultivar Santa Cruz Kada were used in a 4×4 split-plot design with a negative control (sterile distilled water) and a positive control (ASM) at 5 g 100 L^{-1}. The first factor consisted of filtrates of the fungi *P. terrestris, C. inaequalis*, *C. eragrostidis* and *M. echinata*, and the second factor consisted of 0, 5, 10, 15 and 20% concentrations; there was a total of 18 treatments with five replicates, and plots constituted of a plant. Treatments were applied in the 2nd and 3rd true leaves, three days before inoculation with *A. solani* pathogen, when the plants had five leaves. The inoculation was performed in 1 × 10^4 conidia ml^{-1} (Balbi-Penã et al., 2006), and then the plants were kept in a humid chamber in a greenhouse for 12 h.

The severity evaluation of tomato early blight started from the observation of the first disease symptoms in plants. It was severe in leaflets that were treated and inoculated (3rd leaf) and also in those untreated and inoculated (4th leaf), using the diagrammatic scale of Boff (1988). The evaluations were conducted every 5 days for 25 days; there were a total of 5 ratings. For severity evaluation, the area under the disease progress curve (AUDPC) was calculated according to Campbell and Madden (1990). The experiment was conducted in a completely randomized design with four replications. The AUDPC data were submitted to regression analysis ($p<0.05$), with one control and additional treatment.

Biochemical analysis

To evaluate the occurrence of biochemical changes on tomato plants treated with filtrates from saprobes fungi, the specific activity of guaiacol peroxidase, phenylalanine ammonia-lyase and catalase was analyzed.

For biochemical analysis, leaflets were collected from the 3rd leaf (treated and inoculated) to determine the local effect, and also from the 4th leaf (untreated) to observe the systemic effect. The leaves were harvested at 0, 24, 48, 72 and 96 h after inoculation (hpi) with *A. solani* for all treatments, including non-inoculated plants. The leaflets were weighed and stored at -20°C to perform the subsequent biochemical analysis. The enzyme extract was obtained and stored at -20°C to determine the total content of protein and activity of guaiacol peroxidase, phenylalanine ammonia-lyase and catalase.

Total proteins were quantified (Bradford, 1976) and the activity of

guaiacol peroxidase (GPOX) was determined by the method of Lusso and Pascholati (1999); the specific activity was expressed in absorbance units min^{-1} mg^{-1} protein. Catalase activity was determined by Góth method (1991) as adapted by Tomanková et al. (2006) and specific activity was expressed in absorbance unit min^{-1} mg^{-1} protein. The activity of phenylalanine ammonia-lyase enzyme was determined by colorimetric quantification of trans-cinnamic acid released from the substrate of phenylalanine, according to Umesha (2006) and the results were expressed as µmg trans-cinnamic acid min^{-1} mg^{-1} protein.

The results were submitted to statistical method of surface response ($p<0.05$) using SAS statistical software, to determine the mathematical model that best fits the data using the formula $Y = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2$. This mathematical model of equation was used in the Statistica Six Sigma program for building graphics.

RESULTS AND DISCUSSION

In analyzing the influence of different concentrations of saprobe fungi filtrates on the management of early blight in the 3rd leaf, there was a cubic regression model, for all treatments. There was reduction of 89, 83, 92 and 91% of disease at 8, 20, 8, and 7% of the filtrates of *C. eragrostidis*, *C. inaequalis*, *M. echinata* and *P. terrestris*, respectively. Comparing the estimated concentrations of filtrates which promote greater reduction of AUDPC on tomato early blight with ASM, there was a reduction of 79, 63, 81 and 83% in the 3rd leaf with the application of filtrates of *C. eragrostidis*, *C. inaequalis*, *M. echinata* and *P. terrestris*, respectively (Figure 1A).

In the 4th untreated leaf, AUDPC of tomato early blight was adjusted to the cubic regression model when filtrates of *C. eragrostidis* and *C. inaequalis*, *M. echinata* and *P. terrestris* were applied. The lowest values of AUDPC were observed at 8, 7, 7 and 7% with a reduction of 81, 70, 82 and 81%. When compared with the ASM inducer, there was a decrease of 44, 10, 13 and 10% in the 4th leaf with filtrates of *C. eragrostidis*, *C. inaequalis*, *M. echinata* and *P. terrestris*, respectively (Figure 1B).

The application of *C. inaequalis* filtrate on the 3rd leaf (treated) originated a quadratic response in a surface response to the guaiacol peroxidase activity (GPOX activity), catalase (CAT) and phenylalanine ammonia-lyase (PAL) (Figure 2 IA, IIA and IIIA). The influence of the concentrations of filtrates and/or collection time after inoculation with the pathogen is dependent on the enzyme studied.

In the analysis of the activity of GPOX, there was an interaction between the filtrate concentration and time after inoculation with the pathogen. There was a minimum critical point calculated at 48 h with the application of 10% of filtrate; there was reduction in enzymatic activity when compared with untreated plants. Enzyme activity increased at 72 h, with maximum observed at 96 hpi with *A. solani* at all concentrations; although greater one was also observed in untreated plants (Figure 2 IA).

For CAT activity, it was only the effect of time that was observed after inoculation with the minimum and maximum critical points calculated at 28 and 96 hpi, respectively, independent of the filtrate concentration in the treated leaf (Figure 2 IIA). Interaction between time and concentration was observed for PAL activity. There was an increase at 0 h after inoculation and maximum increase was observed at 72 h at 20% concentration; there was 2-fold increase in the enzyme activity compared to untreated plants control (Figure 2 IIIA).

From the analysis of systemic effect on the 4th leaf treated with filtrate of *C. inaequalis,* it can be observed that the GPOX response was adjusted to a linear model and the CAT and PAL enzymes to a quadratic by the surface response method (Figure 2 IB, IIB and IIIB).

Considering the GPOX response, there was an increase in the activity of enzyme after inoculation; it was maximum at 96 h independent of the filtrate concentration from *C. inaequalis* (Figure 2 IB). For CAT, there was concentrations influence, with two maximal activities, one observed at 0 h of inoculation with the pathogen and another 96 hpi, independent of *C. inaequalis* filtrate concentration (Figure 2 IIB). In untreated leaves, the PAL activity increased at 0 h with 2-increase 80 hpi, at estimated concentration of 16% of *C. inaequalis* filtrate (Figure 2 IIIB).

Increase in the activity of both GPOX and CAT was observed (local and systemic) from 72 h in tomato plants treated with filtrate of *C. inaequalis*, after inoculation with *A. solani*. This may be related to the recognition of phytopathogens by the receptors present in the host. These activate a cascade of signaling and transduction of signals which culminate in increasing the activity of these defense enzymes. Such response occurs because the plants have a defense system able to recognize molecular patterns of the pathogen or the one the plant itself originates during the attack (Dubery et al., 2012).

The increase of enzymes such as CAT and GPOX has been reported in many studies about phytopathogen-host interaction, including *A. solani* and *S. lycopersicum.* Here, the defense mechanisms activation in plants can involve the increase of enzymes related to detoxification of reactive oxygen species generated during the plant attack by pathogens, such as GPOX and CAT (Apel and Hirt, 2004). Similar results were found in this study, allowing one to infer that the increase in guaiacol peroxidase does not have a direct relation in the reduction of AUDPC on tomato early blight observed both locally and systemically when the filtrate of *C. inaequalis* was applied. This is because there was an increased pattern of activity of GPOX and CAT in negative control (Figure 1A and B).

The increase in the activity of phenylalanine ammonia-lyase observed locally and systemically (Figure 2 IIIA and IIIB) explains the decrease in tomato early blight (Figure 1A). This increase may be related to the recognition of elicitors present in the filtrate of *C. inaequalis*. According to Henry et al. (2012), in the recognition of elicitors related to pathogen, the plants are also able to notice

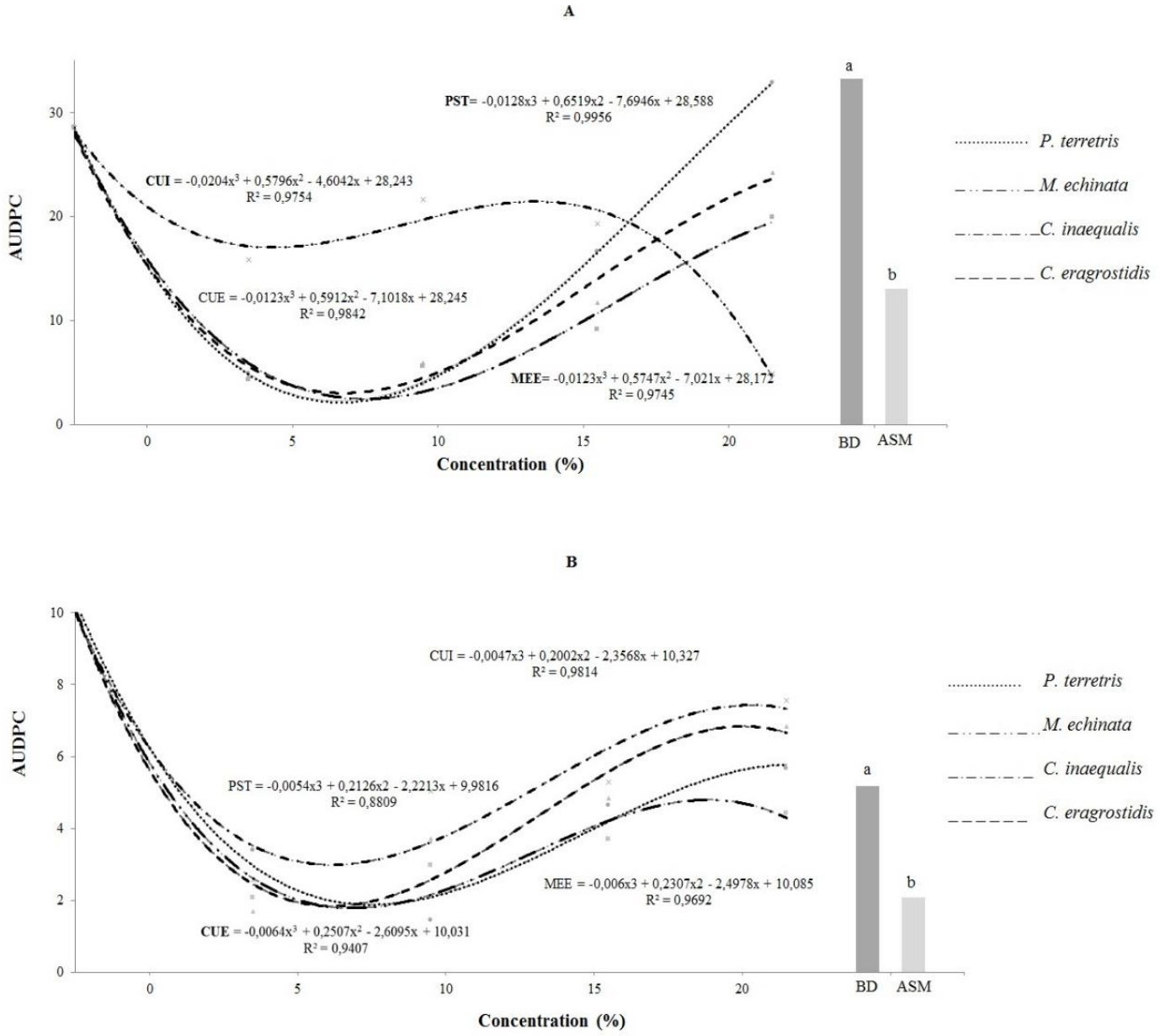

Figure 1. Area under the disease progress curve (AUDPC) of tomato early blight in 3rd leaf-treated (A) and 4th leaf-untreated (B) with concentrations of 5, 10, 15 and 20% of the filtrates from saprobes fungi, Maringa, 2013. *Columns followed by the same letter do not differ by T test at 5% of probability.

those that originate from non-pathogenic microorganisms; they activate resistance mechanisms, such as PAL (Figure 2 IIIA and B).

Akram and Anjum (2011) also found that *Bacillus* strains activate defense responses, by increasing PAL activity and consequently the accumulation of phenolic compounds in tomato plants, which were resistant to the phytopathogen *Fusarium oxysporum.* According to Cavalcanti et al. (2007), the early activation of the defense mechanisms, such as the PAL enzyme, are critical to plant's resistance to pathogen, because it acts by converting the phenylalanine amino acid to trans-cinnamic acid. This conversion starts with carbon channeling from the primary metabolism in phenylpropanoid to the secondary metabolism of plants. This includes salicylic acid formed by the benzoic acid which is also a precursor in the synthesis of several phenylpropanoids such as lignin, coumarins and flavonoids (Lister and Lancaster, 1996). In this study, there was a reduction of early blight of about 63 and 10%, indicating the efficiency of *C. inaequalis* filtrate when tested locally and systemically, respectively.

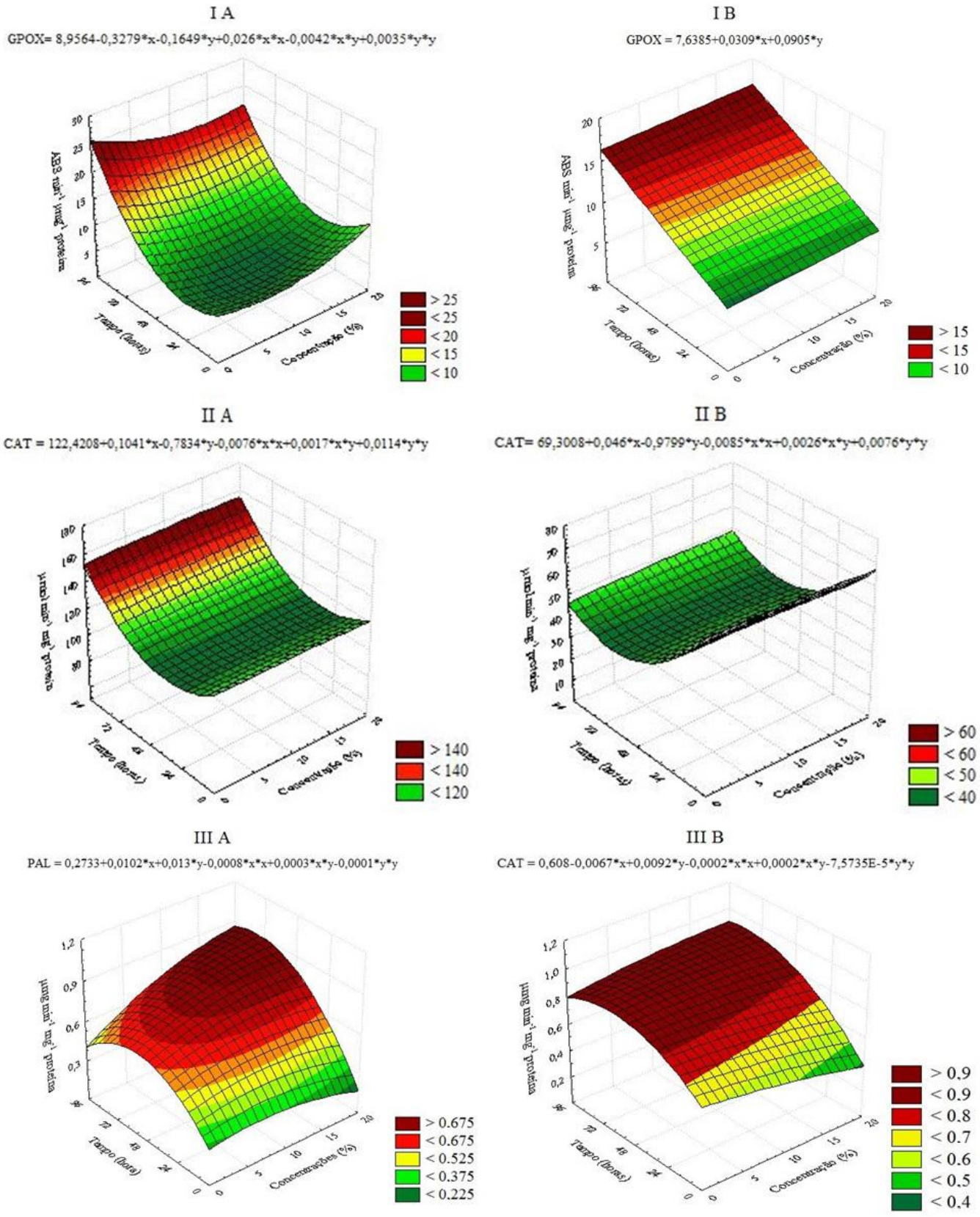

Figure 2. Activity of guaiacol peroxidase (I), catalase (II) and phenylalanine ammonia-lyase in leaves (III) treated - local (A) and untreated - systemic (B) with filtrate concentration of *Curvularia inaequalis,* 72 h before inoculation of *Alternaria solani* and harvested at 0, 24, 48, 72 and 96 hpi, Maringá, 2013.

Studying the effect of the concentrations of *C. eragrostidis* filtrates on treated tomato plants, three days before inoculation on the activity of GPOX, CAT and PAL, there was surface response in a quadratic model, linear and quadratic, respectively, when the samples were collected from the 3rd leaf (treated) (Figure 3 I, II, and II). For the GPOX activity, there was an interaction between the time of harvest after inoculation with *A. solani* and the concentrations of *C. eragrostidis* filtrates applied to the 3rd leaf. There was maximum activity of 96 hpi (concentration of 20%) with two fold increase of enzyme activity compared to control plants (Figure 3 IA).

CAT activity increased proportionally to time after pathogen inoculation; the highest peak of the dependent variable was observed at 96 hpi (Figure 3 IIA). For PAL activity, there was an interaction between the application of *C. eragrostidis* filtrates and the time of collecting the tomato leaves after inoculation with the pathogen. There was maximum enzyme activity of 35 hpi at a concentration of 10%. There was three times increase in the enzyme activity compared to the estimated value of the control plants in the same period (Figure 3 IIIA).

When analyzing untreated plants with filtrate of *C. eragrostidis*, but inoculated with *A. solani*, there was an interaction between the filtrate concentration and collection time in GPOX and PAL activity. However, there was no difference between the filtrate concentration in the CAT activity, where the best fit of regression models was linear, linear and quadratic, respectively (Figure 3 I, II and III B). A dose-dependence was observed with GPOX activity systemically, with three times increase at 20% concentration of *C. eragrostidis* filtrate and 72 hpi with *A. solani* (Figure 3 IB).

For CAT, there was no effect of the concentration on enzyme activity, but time influenced their response, with greater activity from 24 hpi in treated leaves (Figure 3 IIA). For PAL, it was observed an increase in enzyme activity systemically and in proportion to time and filtrate concentration of *C. eragrostidis*; there was duplication of enzyme activity at 96 hpi when 20% of the filtrate was applied (Figure 3 IIIB).

The increase of GPOX and PAL promoted by the filtrate of *C. eragrostidis* can justify the reduction of tomato early blight observed in treated plants (Figure 1A). It may be inferred that the increase of these enzymes can be related to the recognition of elicitor molecules existing in the filtrate by a receptor on the host, both locally and systemically. This is because in both, there was an increase of enzyme activity when the filtrates were applied compared to plants that were untreated but inoculated with the pathogen (Figure 3 IA and IB). Both enzymes, GPOX and PAL have been reported in defense of plants against pathogen attack and may be activated simultaneously when the plant is exposed to an elicitor agent. Chmielowska et al. (2008) verified that seedlings of bean and lupine when treated with copper, but not inoculated with pathogens had a higher activity of peroxidase and phenylalanine ammonia-lyase.

The activation of peroxidase is associated with the primary events of the resistance mechanisms induced by elicitors. This is because the enzyme acts on hydrogen peroxide detoxification formed during the oxidative explosion caused after recognition of elicitors during the defense system activation (Van Loon et al., 2006). This generation of hydrogen peroxide in elicited cells increases the peroxidase activity involved in the cross oxidative connection of structural proteins in the cell wall rich in the repetition of proline. It also increases tyrosine that strengthens the plant's cell walls, slowing the phytopathogen colonization process (Kishor et al., 2005).

An increase in the PAL activity in plants treated with filtered *C. eragrostidis* may have an essential role in reducing the AUDPC of tomato early blight. According to Umesha (2006), phenylalanine ammonia-lyase is crucial in the protection of tomato plants against the attack of *Clavibacter michiganensis* subsp. *michiganensis*. The enzyme activity is related to the plant resistance to pathogens, essentially, for being involved in the first step of the phenylpropanoids synthesis and its conversion to trans-cinnamic acid, resulting in compounds with antimicrobial activity. This is besides lignin increment, which gives greater resistance to the plants' cell wall (Nakazawa et al., 2001).

In Figures 4 IA, IIA and IIIA, there was a significant effect ($P<0.05$) in the interaction between filtrate concentration of *P. terrestris* and harvest time for determining guaiacol peroxidase, catalase and phenylalanine ammonia-lyase, with best fit in the quadratic model for the three enzymes when analyzed locally (3rd leaf).

GPOX activity was reduced in the gap between 5 and 15% of the filtrate and opposite (Figure 4 IA). However, there was an increase of CAT within the same interval of concentrations; 96 hpi increase in 10 % concentration, where there was 3-fold increase in enzyme activity. PAL was not influenced by the application of *P. terrestris* filtrate concentrations; however enzyme activity increased with time after inoculation with *A. solani* (Figure 4 IA).

For the GPOX activity in 4th leaf (untreated), there was maximum increment of the enzyme in plants that were not treated with *P. terrestris* filtrate, 96 h after inoculation (Figure 4 IB). CAT activity increased at 72 hpi when 15% was applied, with 2-fold increase in GPOX activity (Figure 4 IIB). For PAL quantification in the untreated leaves, the greatest activity was observed at 96 hpi independent of the filtrate concentration (Figure 4 IIIB).

The reduction of AUDPC in tomato early blight (Figures 1A and B) might be correlated to an increase of CAT and PAL activity in plants treated with filtrates of *P. terrestris*. Cavalcanti et al. (2006) observed that tomato plants, from the group Santa Cruz Kada, previously induced with an elicitor made of necrotic tissue extract with *Xanthomonas vesicatoria*, presented less bacterial spot severity caused by *X. vesicatoria.* This protection was related to the

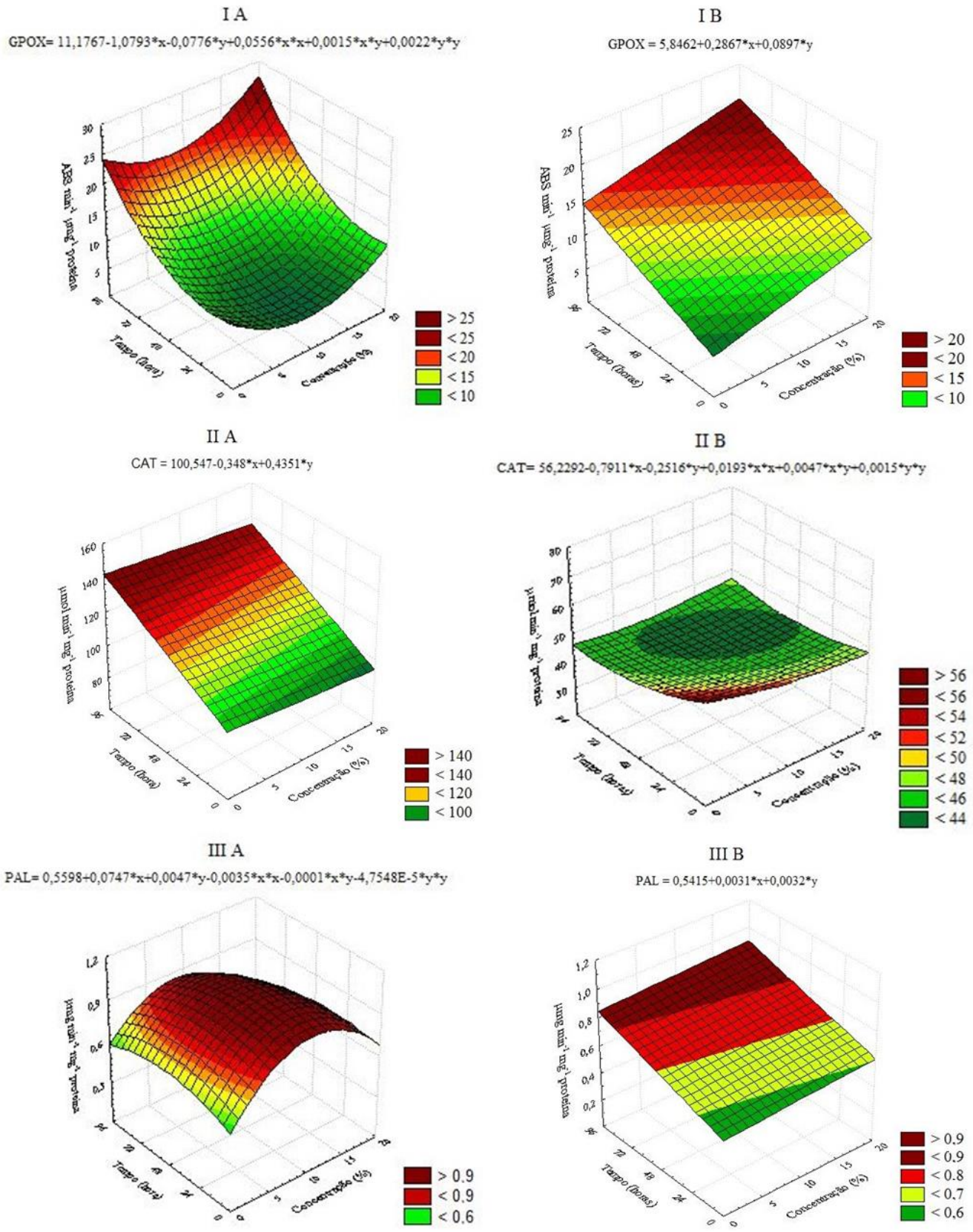

Figure 3. Activity of guaiacol peroxidase (I), catalase (II) and phenylalanine ammonia-lyase in leaves (III) treated - local (A) and untreated - systemic (B) with filtrates concentration of *Curvularia eragrostidis* 72 h before inoculation of *Alternaria solani* and harvested at 0, 24, 48, 72 and 96 hpi, Maringa, 2013.

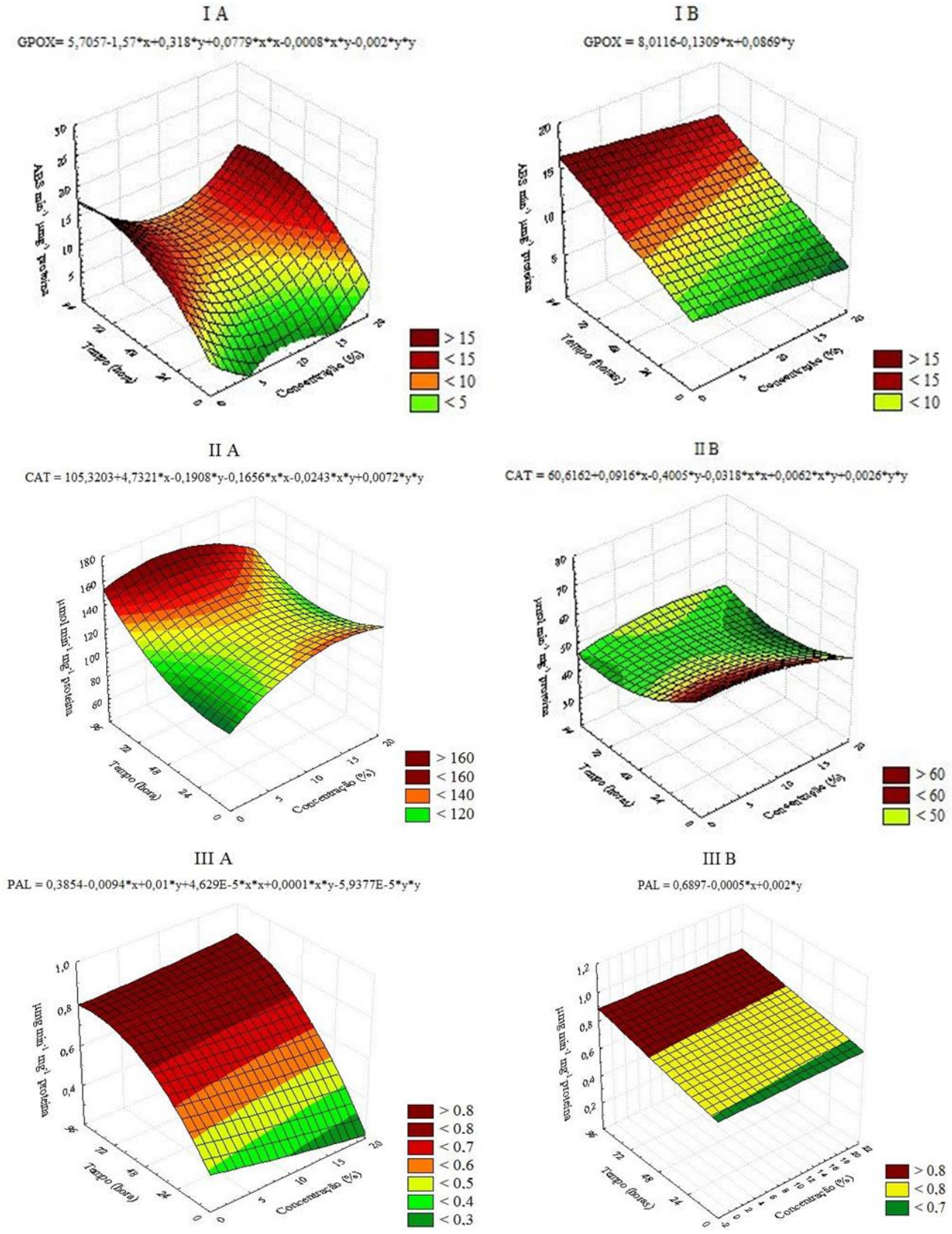

Figure 4. Activity of guaiacol peroxidase (I), catalase (II) and phenylalanine ammonia-lyase in leaves (III) treated - local (A) and untreated - systemic (B) with filtrates concentration of *Pseudobotrytis terrestris* 72 h before inoculation of *Alternaria solani* and harvested at 0, 24, 48, 72 and 96 hpi, Maringa, 2013.

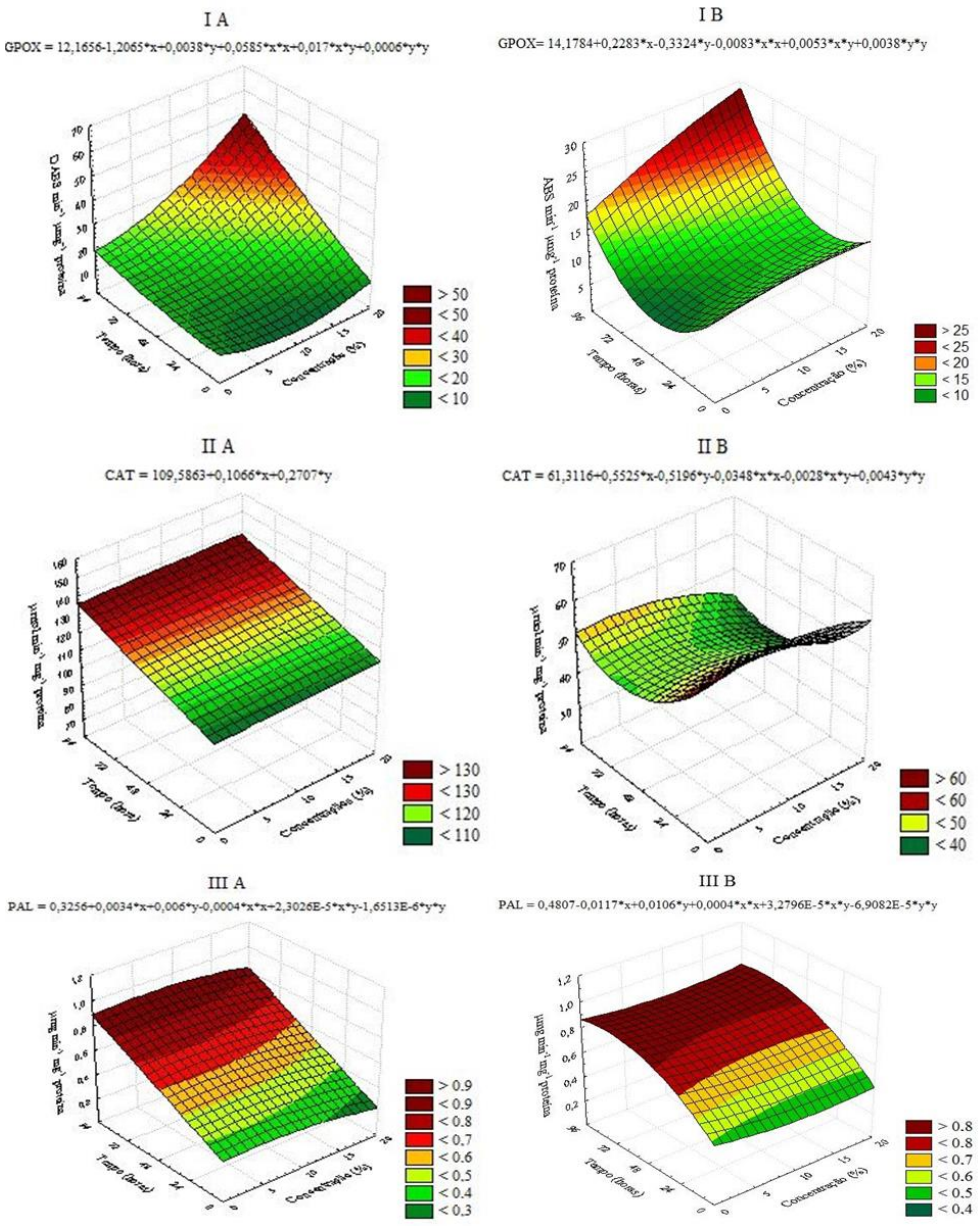

Figure 5. Activity of guaiacol peroxidase (I), catalase (II) and phenylalanine ammonia-lyase in leaves (III) treated - local (A) and untreated - systemic (B) with filtrates concentration of *Memnoniella echinata* 72 h before inoculation of *Alternaria solani* and harvested at 0, 24, 48, 72 and 96 hpi, Maringa, 2013.

increase in catalase activity from 24 h with peak at72 hours after the treatments. This similarly occurred with the application of P. *terrestris* filtrate in the interaction of *A. solani* and *S. lycopersicum* (Figure 4 IA).

The enzyme catalase acts in the dismutation of two H_2O_2 molecules into water and oxygen (Sharma et al., 2012); thus, the hydrogen peroxide accumulation can be estimated indirectly by the action of this enzyme. H_2O_2 has been applied in various stress conditions, including induction through biotic and abiotic inductors where CAT rapidly degrades H_2O_2 in an efficient manner (Mallick and Mohn, 2000). This detoxification is dependent on the intensity, duration and type of elicitation (Han et al., 2009).

The equation model of quadratic regression had the best fit for the interaction between the filtrate concentration of *M. echinata* and time of harvest after inoculation with *A. solani* for GPOX activity, using the surface response method and the linear method for CAT and PAL, when determined locally (Figure 5 IA, IIA and IIIA).

GPOX increased according to the filtrate concentration and the time after inoculation, with 3-fold increase in

enzyme activity after 96 hpi at a 20% concentration in relation to treatment with control plants (Figure 5 IA). CAT activity increased linearly with time of harvest. However, it was not influenced by concentration (Figure 5 IIA). While the application of *M. echinata* filtrate reduced PAL activity in 3rd leaf by increasing the filtrate concentration and the harvesting time after pathogen inoculation (Figure 5 IIIA).

Systemically, the enzymes guaiacol peroxidase, catalase and phenylalanine ammonia-lyase were adjusted to the quadratic model (Figure 5 IB, IIB and IIIB). GPOX activity increased with concentration and harvesting time after inoculation with 2-fold increase by applying the filtrate at 20% and 96 hpi, compared to activity of leaves collected from untreated plants at the same period (Figure 5 IB). The CAT was affected with the time of harvest and there was reduction in the activity with *A. solani* infection, independent of the filtrate concentration of *M. echinata* (Figure 5 IIB).

The application of concentrations of *M. echinata* filtrate promoted greater GPOX activity; this indicates a possible reduction of tomato early blight leads to increase in the activity of enzymes. The increase in peroxidase activity may also be associated with an increase of structural proteins synthesis, which strengthens the cell wall, acting as a barrier to pathogen (Van Loon et al. 2006).

By applying filtrates of *C. inaequalis*, *C. eragrostidis*, *P. terrestris* and *M. echinata*, it can be observed that they are capable of reducing AUDPC of tomato early blight through the induction of distinct biochemical mechanisms. The filtrates of these fungi acted as elicitors agents, being recognized by receptors present in tomato plants, activating its defensive enzymes with increased GPOX, CAT and PAL. The plants' multicomponent defense response is directly related to interactions between the elicitor and receiver, with amplitude, duration and quality dependent on the specific signaling generated (Brencic and Winans, 2005). The knowledge of this variation may explain the distinct behavior observed in each filtrate from the species of saprobes fungi presented in this work.

The peaks of activity from GPOX and CAT simultaneously are noticed when the filtrates of saprobes are applied on the tomato plants. Interestingly, GPOX and CAT showed opposing activities to each other, that is, when there was an increase of GPOX activity the CAT activity was reduced and vice versa. Such response can be observed clearly in plants treated with *P. terrestris*, in which there was a reduction in the peroxidase activity between the concentrations of 5 and 15%; the catalase increased between the same concentrations. Because the enzymes compete for the same substrate, the H_2O_2 generated during oxidative explosion is caused by stress (Sen et al., 2003).

When considering the enzymes activation related to pathogenesis, it may be observed that the filtrate of *M. echinata* induced GPOX activity in untreated leaves and *C. eragrostidis* increased the activity of PAL and GPOX, systemically. The potential to activate the defense responses in parts of the plants that were untreated is a characteristic of great importance for resistance inducer, because it prepares the plant tissue that has not received the treatment for a possible pathogen attack. Systemic activation is normally regulated by a signaling cascade mediated by H_2O_2, salicylic acid, MAPKs and/or other molecules after recognition of elicitor molecule by receptors present in the cell membrane of the plant cell (Conrath et al., 2006).

In conclusion, the filtrates from *C. eragrostidis*, *C. inaequalis*, *M. echinata* and *P. terrestris* promoted reduction of AUDPC in tomato early blight in greenhouse conditions, when applied three days before inoculation locally and systemically. New studies may be performed to identify elicitor molecule(s) and can be used in the future in the management of plant diseases.

Conflict of Interests

The authors have not declared any conflict of interests.

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