Article

Activation of defense mechanisms in pyre corn through the use of Microgeo® organic fertilizer

David Fernando Posso Suárez
Amanda do Prado Mattos§
Bruna Broti Rissato
Kátia Regina Freitas Schwan-Estrada

Department of Agronomy-State University of Maringá (UEM). Av. Colombo 5790, Zone 7, Maringá, Brazil. PR. 87020-900.

§Corresponding author: pradomattosa@gmail.com.

Abstract

The induction of resistance (IR) in plants by natural products has been the subject of several studies, these being activated by eliciting molecules of biotic or abiotic origin. Elicitors activate resistance mechanisms such as pathogenesis-related proteins (PRP's), which act to protect plants by preventing pathogen development. The present study aims to verify the activation of PRP's (peroxidase, catalase, polyphenoloxidase and β-glucanase) in pyre corn plants (*Zea mays*) treated with different concentrations (0%, 0.5%, 1%, 1.5%, 3% and 5%) of the commercial biofertilizer Microgeo®. Analyzing the PRP's enzymes, it was observed that the Microgeo® activated and increased the activity of these enzymes and that this induction is more related to the activation time, but not to the concentration used.

Keywords: defense mechanism, enzymatic activity, induced resistance, inducers.

Reception date: April 2020
Acceptance date: July 2020

Introduction

Corn cultivation is affected by pests and diseases, which are controlled through the indiscriminate use of pesticides, an activity that causes contamination of the environment and people, in addition to the selection of resistant races of pathogens (Ghini and Kimati, 2002; Casela *et al.*, 2006; Costa *et al*., 2012; Cota *et al*., 2013; Wordell-Filho *et al*., 2017). An alternative for disease control in corn cultivation is resistance induction, widely studied in the area of plant pathology (Roncatto and Pascholati, 1998; Silva *et al*., 2008; Krzyzaniak *et al*., 2018, Lorenzetti *et al*., 2018).

Pascholati and Dalio (2018) report that resistance induction is the activation of the defense systems of the plant, when they come into contact with the elicitors, through the perception and transduction of biological signals at the cellular level. Angelova *et al*. (2006), describe elicitors as substances of biotic origin, as viable organisms and components of their structures, or of an abiotic type such as salicylic acid, phosphites, silicates, among others.

Biofertilizers that act as powerful elicitors due to the biotic and abiotic diversity of their final composition that can activate defense reactions in the plant are also considered resistance inducers (Barros *et al*., 2010). A characteristic of resistance inducers is that they do not act in the same way as conventional agrochemicals, since they present low toxicity to the pathogen; however, they have the ability to activate latent defense mechanisms in plants (Colares and Bonaldo, 2014).

The use of inducers for the activation of resistance mechanisms in different crops is a sustainable strategy with high potential in the control of diseases that can be adopted in corn cultivation. The objective of this study is to verify the induction of proteins related to the pathogenesis, peroxidase, catalase, β-1,3 glucanase and polyphenoloxidase, in plants of pyre maize (*Zea mays*) Everta genetic group treated with different concentrations of the organic fertilizer Microgeo®.

Materials and methods

The experimental phase was carried out at the Laboratory of Alternative Control and Resistance Induction of the Maringa State University in Parana, Brazil between the months of June 2017 to June 2018.

Resistance inductor

To evaluate the induction of proteins related to pathogenesis in pyre maize, the biofertilizer Microgeo® was used, which contains biodynamic preparations, recalcitrant substances, pentoses, minerals and bran in its composition. Microgeo® is a product that has balanced components that nourishes, regulates and maintains the continuous production of biological fertilizers; through the continuous liquid composting process (CLC).

In a greenhouse, in 3 L pots, in a substrate made up of two parts of sterilized soil, with one part of sterilized sand (2:1), three seeds of pyre maize variety Para were sown, for a total of six treatments and eight repetitions per treatment. At 10 days after germination, the pyre corn plants were treated with Microgeo®. 100 ml of the different concentrations were prepared (0%; 0.5%; 1%; 1.5%; 3% and 5%) and with a single foliar application, eight plants were sprayed per treatment.

Until drainage point, the treated plants remained in the greenhouse for a period of seven days. Plants treated with distilled water were used as a control. Every 24, 48, 72, 96 and 120 hours after the application of the Microgeo® in the different concentrations, a leaf was removed, washed in distilled water and kept on ice and then weighed on a precision balance.

Preparation of the enzyme extract

The collected tissue was macerated in mortar with liquid N_2 and homogenized in 50 mM phosphate buffer and pH 7 + 0.1 mM EDTA. Subsequently, the homogenate was transferred to two microcentrifuge tubes (2 ml) per sample, which were centrifuged for 30 min at 12 000 g/4 ºC, the obtained supernatant was transferred to previously labeled microcentrifuge tubes (2 ml) and stored at -20 ºC until the moment of quantification.

Enzymatic analysis

The enzymatic analyzes were performed in duplicate (2 microcentrifuge tubes with 2 ml of enzyme extract). The enzymatic extract was used to evaluate the content of total proteins and of the enzymes peroxidase, catalase, polyphenoloxidase and β-1.3 glucanase.

Total proteins

For the determination of total proteins, the Bradford method (1976) was used, which measures the interaction between the high molecular weight protein and the Coomassie Brilhant Blue -BG-250 dye. In 50 µl of enzyme extract, 2.5 ml of Bradford was added and vortexed. After 5 min, the reading was made in a glass cuvette at 595 nm in a spectrophotometer.

50 µl of distilled water with 2.5 ml of Bradford was used as blank. The protein concentration of each sample was expressed mg protein sample ml^{-1}, whose absorbance was extrapolated in the protein standard curve (ASB) (Y= 0.845x -0.0227). Where: Y= is the absorbance at 595 nm and X the protein concentration for each 0.5 mg mL^{-1} of bovine serum albumin.

Guayacol peroxidase activity

Peroxidase activity was determined at 30 ºC; through the direct spectrophotometric method, by measuring the conservation of guaiac in tetraguayacol at 470 nm (Lusso and Pascholati, 1999). The solution mixture consisted of 2.9 ml of enzyme substrate, 306 µl of hydrogen peroxide, 250 µl of 2% guaiacol and 87.5 ml of 0.01 M phosphate buffer (pH 6) and 100 µl of enzyme extract.

The reaction was followed in a spectrophotometer at 470 nm, for a period of 2 min. Activity was determined by the variation that occurred between the extreme values located in the linear increase interval and expressed in ABS min^{-1} mg^{-1} protein.

Catalan activity

Catalase activity was determined by the stable complex formed by ammonium molybdate with hydrogen peroxide. A 100 µl aliquot of the enzyme extract was incubated in 0.5 ml of reaction mixture containing 60 mM hydrogen peroxide in 60 mM potassium phosphate buffer (pH 7.4) at 38 °C for 4 min. After this period, 0.5 ml of 32.4 mM ammonium molybdate was added to stop the reaction and stop the consumption of hydrogen peroxide by the enzyme in the extract.

A blank for each sample was also prepared, by adding ammonium molybdate to the reaction mixture, regardless of the incubation period. The reading of the yellow complex formed by the molybdate and hydrogen peroxide was quantified at 405 nm in a spectrophotometer.

The difference between the absorbance of the blank and the incubated sample determined the amount of hydrogen peroxide used by the enzyme. The H_2O_2 concentration was determined using the extinction coefficient €= 0.0655 mM cm^{-1} and expressed in µmol min^{-1} mg^{-1} of protein.

Polyphenoloxidase activity

Polyphenoloxidase activity was determined by the methodology of Dungmal and Apenten (1999), to quantify the oxidation of the catechol converted to quinone, a reaction measured by the polyphenoloxidase enzyme. The enzyme substrate consisted of a 20 mM concentration of catechol, dissolved in 100 mM potassium phosphate buffer (pH 6.8).

The reaction was conducted at 30 °C, by the addition of 100 µl of enzyme extract in 900 µl of the substrate, for subsequent reading in a 420 nm spectrophotometer, by the direct method, for 2 min. Activity was determined by the variation occurred between the extreme values located in the linear increase interval and expressed in unit min^{-1} mg^{-1} protein.

B-1,3 glucanase activity

The β-1.3 glucanase activity was determined by the colorimetric quantification of reducing sugars released with laminarin (Vogelsang and Braz, 1993). For this, 150 µl of the enzyme extract and 150 µl of laminarin (1.5 mg ml^{-1}) in extraction buffer were added. As a control, the same reaction was used, only that the laminarin was placed before incubation.

Incubation for the reaction was maintained at 40 °C for 60 min in a water bath. After the incubation period, a 30 µl aliquot was removed from the incubated tubes in addition of 1.5 ml of PAHBAH (0.5 g of 4-Hydroxybenhydrazide diluted in 20 ml of 0.5 M HCl, and increased with 80 ml of 0.5 M NaOH), whose mixture was kept in a water bath at 100 ºC for 5 min and then cooled in an ice bath.

The reducing sugars formed were quantified by the Lever method (1972). The absorbance reading was determined at 410 nm, in a spectrophotometer, discounting the absorbance values of the blank, being that the amount of sugars was determined using the standard glucose curve (Y= 1.1512x + 0.205). Where: Y= is the absorbance at 410 nm and x= the concentration of reducing sugars expressed in mg of glucose min^{-1} mg^{-1} of protein.

Statistical analysis

Each experimental unit was represented by a pot with three pyre corn plants. The assays for enzyme analysis were conducted in duplicate, with a completely randomized outline with eight replications. The assumptions of the homogeneity of the variances and the distribution of the dice were taken care of, by the Levene and Shapiro Wilk tests, respectively.

The results obtained were subjected to analysis of variance ($p< 0.05$) by the F test and the means of the treatments were analyzed by the Tukey test and subsequent regression analysis to evaluate the different doses of the treatments. The statistical analysis program and planning of experiments SISVAR 5.6 was used (Ferreira, 2014).

Results and discussion

Catalase

For the specific catalase activity, the regression was significant for the cubic model. Three times more catalase enzyme activity (44.3 µmol min^{-1} mg^{-1} protein) was observed in the first 24 h in the concentration of 3.7% Microgeo® compared to the control treatment, which were the plants that were treated only with distilled water (Figure 1A).

For the 48 hours, the maximum activity of the enzyme was observed in the 1.87% concentration with 51 µmol min^{-1} mg^{-1} protein, 2.5 times more than the control (Figure 1B). For the 72 h, the maximum activity of the enzyme was observed in the 4.5% concentration with 63 µmol min^{-1} mg^{-1} protein, value 3.3 times greater than the treatment with water (Figure 1C).

Sampling of leaves at 96 h, with a concentration of 3.9% Microgeo®, showed a higher catalase activity of 56 µmol min^{-1} mg^{-1} protein, a result 2.6 times higher than treatment with water (Figure 1D). In the 120 h schedule, 64 µmol min^{-1} mg^{-1} protein was observed in the 1.3% concentration, a value 4 times higher than the treatment with water (Figure 1E).

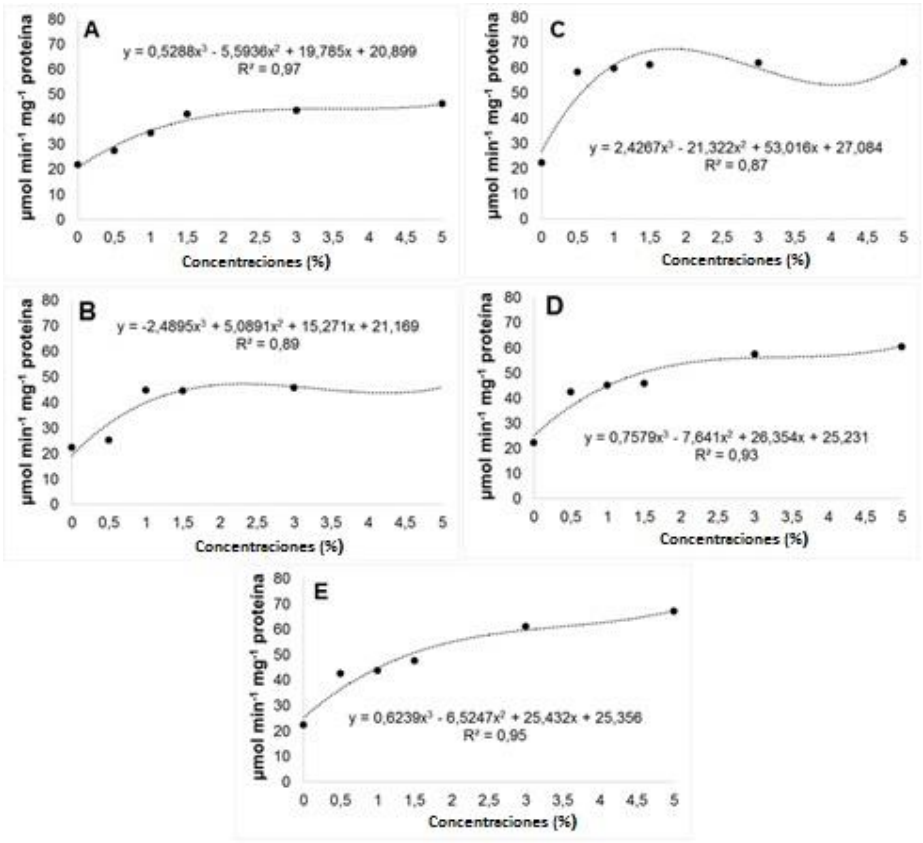

Figure 1. Specific catalase activity in pyre maize plants as a function of the splitting of the interaction between the different concentrations of Microgeo® within the following leaf sampling times: 24 h (A); 48 h (B); 72 h (C); 96 h (D); and 120 h (E); CV= 3.45%.

In all the Microgeo® concentrations, except 0%, they induced the specific activity of catalase (Table 1). At 72 h after application of the 0.5, 1, 1.5% and 3% concentrations, there was the greatest increase in enzyme activity, while at the 5% concentration an increase in activity occurred 120 h after the application.

Table 1. Specific activity of catalase (µmol min^{-1} mg^{-1} protein) in pyre corn plants as a function of the doubling of the interaction between leaf sampling times within Microgeo® concentrations.

Schedule (h)	Concentrations (%)					
	0	0.5	1	1.5	3	5
24	22.03 a	27.59 c	34.51 c	42.23 d	43.48 c	46.19 c
48	22.26 a	25.26 c	44.67 c	44.65 cd	45.19 c	45.94 c
72	22.4 a	58.45 a	59.75 a	61.24 a	61.9 a	62.13 b
96	22.42 a	42.44 b	44.97 a	45.69 bc	57.51 b	60.5 b
120	22.52 a	42.45 b	43.82 b	47.69 c	61.05 a	67.2 a
CV (%)	3.45					

Means followed by the same letter in the columns do not differ significantly from each other by the Tukey test ($p<$ 0.05).

Guaiac peroxidase

In the specific peroxidase activity, it was observed by the doubling of the concentrations within the leaf sampling times, that the regression model was significant with cubic adjustment (Figure 2). At the 2.4% concentration of Microgeo®, 1.6 times more activity of the peroxidase enzyme (23.24 ABS min^{-1} mg^{-1} protein) was observed when compared to the treatment with water, at 24 h (Figure 2A).

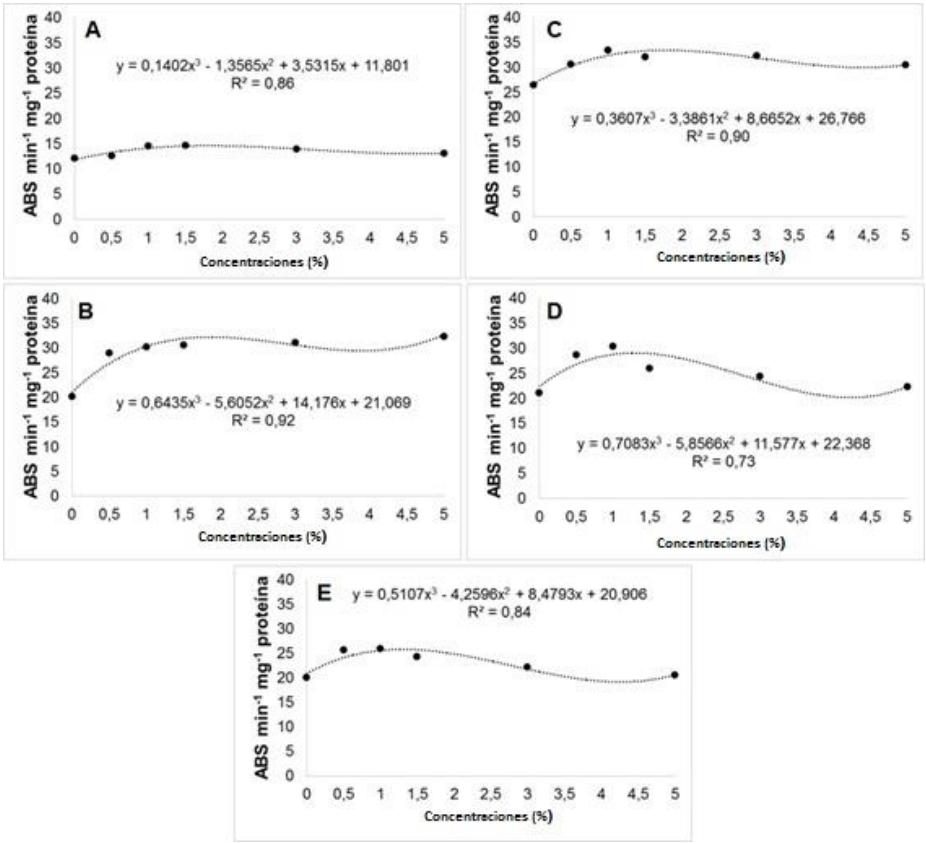

Figure 2. Specific peroxidase activity in pyre corn plants as a function of the splitting of the interaction between the different concentrations of Microgeo® within the following leaf sampling times: 24 h (A); 48 h (B); 72 h (C); 96h (D); and 120 h (E); CV= 7%.

In the 48 hr schedule, the maximum peroxidase activity was observed in the 1.7% concentration with 22.97 ABS min^{-1} mg^{-1} protein, 1.1 times greater than the treatment with water (Figure 2B). In the 72 h schedule, the maximum enzymatic activity of peroxidase was observed in the concentration of 1.7% with 47.68 ABS min^{-1} mg^{-1} protein, value 1.6 times greater than treatment with water (Figure 2C).

At the 3% concentration, an enzymatic activity of 34 ABS min^{-1} mg^{-1} protein was observed, a value 1.6 times greater than treatment with water (Figure 3D). At 120 h, 46.84 ABS min^{-1} mg^{-1} protein was observed in the concentration of 2.4%, a value 2.8 times higher than the control treatment.

All Microgeo® concentrations, except 0%, induced specific peroxidase activity (Table 2). 72 h after application in concentrations 0.5; one; 1.5 and 3%, there was the greatest increase in enzyme activity and at 5% concentration there was an increase in activity 120 h after application.

Table 2. Specific activity of peroxidase (ABS min^{-1} mg^{-1} protein) in pyre corn plants as a function of the doubling of the interaction between leaf sampling times within Microgeo® concentrations.

Schedule (h)	Concentrations (%)					
	0	0.5	1	1.5	3	5
24	14.4 d	16.02 c	21.6 d	22.65 c	22.75 c	23.02 c
48	19.92 bc	22.35 b	22.4 cd	22.7 bc	22.78 c	23.43 c
72	29.42 a	44.58 a	44.55 a	44.98 a	44.96 a	42.71 b
96	21.95 b	23.83 b	25.94 c	33.14 b	33.2 b	39.41 b
120	16.25 cd	23.6 b	31.98 b	45.06 a	49.21 a	82.69 a
CV (%)	7					

Means followed by the same letter in the columns do not differ significantly from each other by the Tukey test ($p<$ 0.05). Enzymatic activity, inducers, defense mechanism and induced resistance.

Polyphenoloxidase

The doubling of the concentrations within the leaf sampling times was significant for the cubic regression model when analyzing polyphenoloxidase (Figure 3). At the 1.8% concentration of Microgeo®, 1.3 times more activity of the enzyme polyphenoloxidase (3.4 unit min^{-1} mg^{-1} protein) was observed.

When compared to 24 h water treatment (Figure 3A). In the 48 hr schedule, the maximum activity of polyphenoloxidase was observed in the 4.2% concentration with 3.6 unit min^{-1} mg^{-1} protein, value 1.1 times greater than the treatment with water (Figure 3B).

In the 72 h schedule, the maximum enzymatic activity was observed at a concentration of 1.7% with 9.3 units min^{-1} mg^{-1} protein, value 2.5 times higher than treatment with water (Figure 3C). In the 3% concentration, an enzymatic activity of 4.2 unit min^{-1} mg^{-1} protein was observed, 1.2 times higher value than treatment with water (Figure 3D). In the 120 h schedule, 4.53 unit min^{-1} mg^{-1} protein was observed in the 5% concentration, 1.2 times greater than the control treatment.

In all the Microgeo® concentrations, except 0%, they induced specific activity of polyphenoloxidase (Table 3). In the 72 h after the application of the concentrations of 0.5%, 1%, 1.5% and 3%, there was the greatest increase in the activity of the enzyme, while at the concentration of 5% there was an increase in the activity of 120 h after the application.

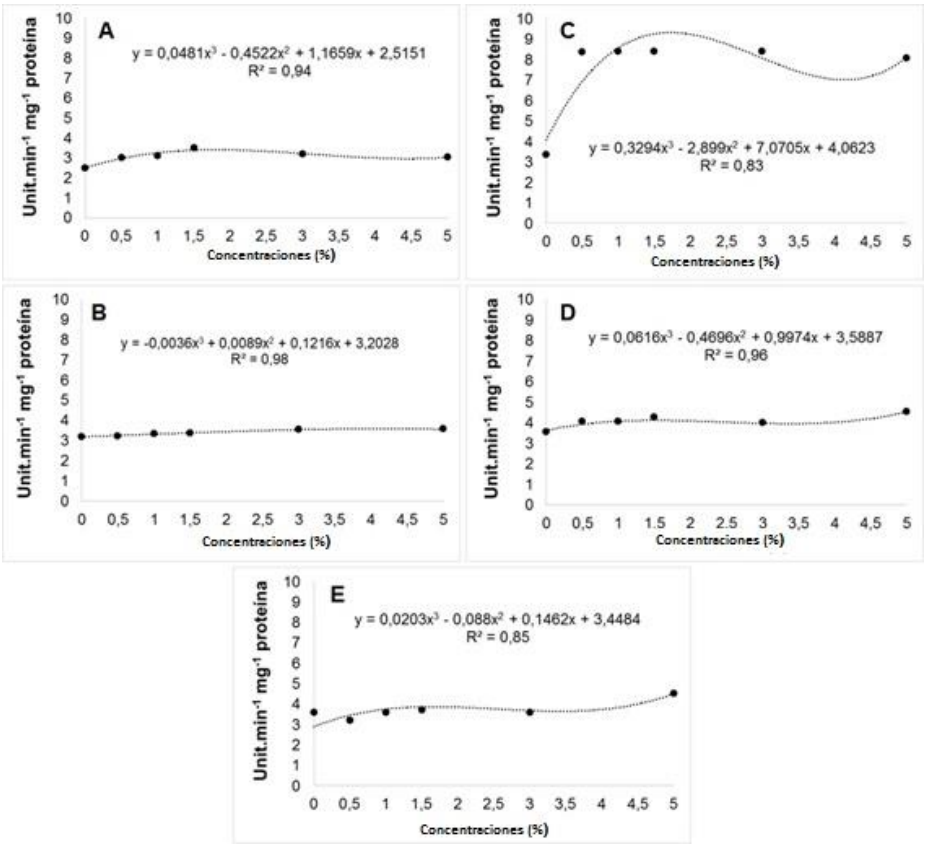

Figure 3. Specific activity of polyphenoloxidase in pyre corn plants as a function of the splitting of the interaction between the different concentrations of Microgeo® within the following leaf sampling times: 24 h (A); 48 h (B); 72 h (C); 96 h (D); and 120 h (E); CV= 7%.

Table 3. Specific activity of polyphenoloxidase (unit min^{-1} mg^{-1} protein) in pyre corn plants as a function of the doubling of the interaction between leaf sampling times within Microgeo® concentrations.

Schedule (hours)	Concentrations (%)					
	0	0.5	1	1.5	3	5
24	2.52 b	3.03 c	31.4 c	3.52 b	3.22 b	3.05 c
48	3.21 ab	3.24 bc	3.36 bc	3.38 b	3.55 b	3.58 c
72	3.37 ab	8.36 a	8.39 a	8.4 a	8.4 a	8.07 a
96	3.56 a	4.07 b	4.08 b	4.27 b	4.02 b	4.54 b
120	3.05 a	3.2 bc	3.58 bc	4.22 b	3.58 b	4.53 b
CV (%)	10.83					

Means followed by the same letter in the columns do not differ significantly from each other by the Tukey test ($p<$ 0.05).

B 1.3-glucanase

The doubling of concentrations within leaf sampling times was significant for the cubic regression model when analyzing β 1.3-glucanase (Figure 4). At the 1.8% concentration of Microgeo®, 1.3 times more activity of the enzyme glucanase (3.4 mg glucose min^{-1} mg^{-1} protein) was observed when compared to the treatment with water at 24 h (Figure 4A).

At 48 h the maximum glucanase activity was observed in the 4.2% concentration with 3.6 mg glucose min^{-1} mg^{-1} protein, result 1.1 times greater than the treatment with water (Figure 4B). In the 72 hour time, the maximum activity of this enzyme was observed in the concentration of 1.7% with 9.3 mg glucose min^{-1} mg^{-1} protein, value 2.5 times higher than treatment with water (Figure 4C).

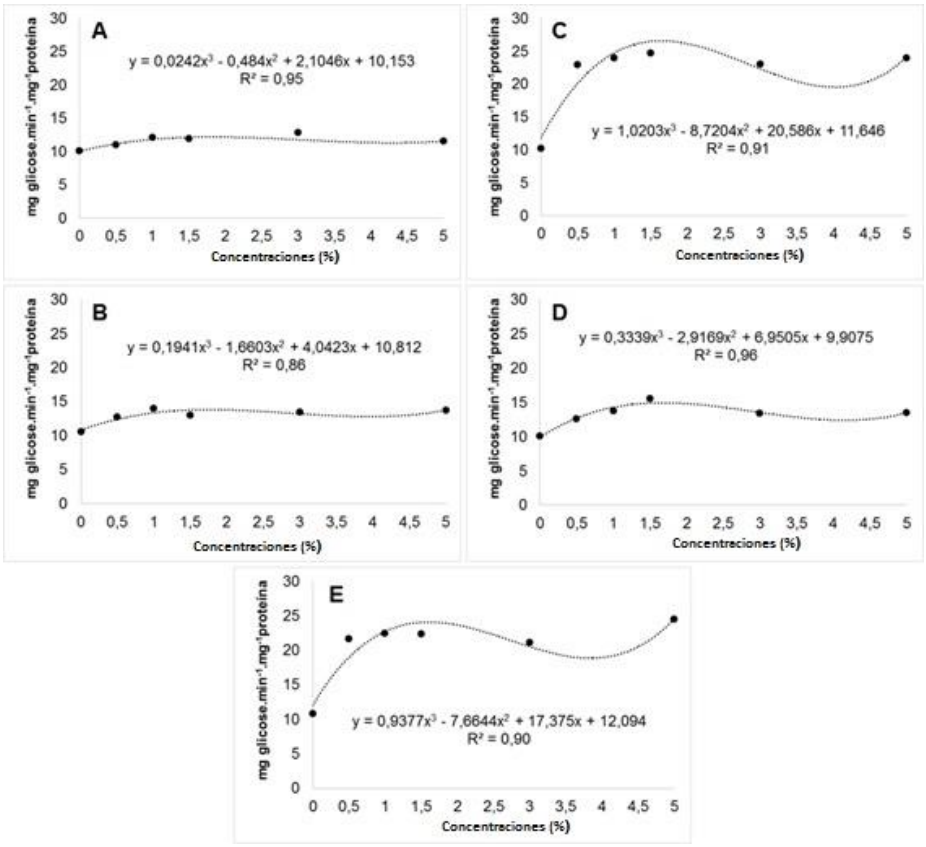

Figure 4. Specific activity of β 1.3 glucanase in pyre corn plants as a function of the splitting of the interaction between the different concentrations of Microgeo® within the following leaf sampling times: 24 h (A); 48 h (B); 72 h (C); 96 h (D); and 120 h (E); CV= 7%.

In the 3% concentration, an enzymatic activity of 4.2 mg glucose min^{-1} mg^{-1} protein was observed, 1.2 times higher than that of water treatment (Figure 4D). At 120 h, 4.53 mg glucose min^{-1} mg^{-1} protein was observed in the 5% concentration, 1.2 times higher than the control treatment (Figure 4E).

All the Microgeo® concentrations, except 0%, induced specific glucanase activity, with a peak of activity 72 h after the application of the concentrations 0.5%, 1%, 1.5%, 3% and 5% and another 120 h after the application (Table 4).

Table 4. Specific activity of β 1.3-glucanase (mg glucose min^{-1} mg^{-1} protein) in pyre corn plants as a function of the splitting of the interaction between leaf sampling times within Microgeo® concentrations.

Schedule (h)	Concentrations (%)					
	0	0.5	1	1.5	3	5
24	10.11 a	11.05 b	12.14 b	11.98 c	11.83 b	11.59 b
48	10.58 a	12.7 b	13.97 b	13.02 bc	13.43 b	13.75 b
72	10.22 a	23.94 a	23.97 a	24.74 a	23.11 a	24.02 a
96	10.06 a	12.57 b	13.77 b	15.5 b	13.38 b	13.5 b
120	10.8 a	21.66 a	22.49 a	22.42 a	21.17 a	24.48 a
CV (%)	7					

Means followed by the same letter in the columns do not differ significantly from each other by the Tukey test ($p< 0.05$).

In general, it was observed that the increase in the activity of the enzymes catalase, peroxidase, polyphenoloxidase and β-1.3-glucanase, started 24 h after the application of Microgeo®, mainly in the doses of 1 and 1.5%, so that the more intense enzymatic activity was registered between 72 and 96 h after the application of the treatments. The catalase activity was observed 24 h after treating the plants with the different concentrations of Microgeo®, with the highest activities recorded at 72 and 96 h at the 1% and 1.5% concentrations.

Similar results were found by Sytykiewicz (2015) confirming significant alterations in the catalase gene transcription responses in different corn hybrids, when they were exposed to herbivores by aphids or as Lanubile *et al*. (2012), who found that the ears of corn inoculated with *Fusarium verticilloides,* protect themselves against oxidative stress and against the attack of the pathogen, inducing greater enzymatic activity of catalase.

In the peroxidase enzyme activity, it was observed that its activity started 24 h after the plants were treated, the highest activities being recorded between 48 and 72 h; however, peroxidase activity was also observed in untreated plants. Similar results were obtained by Cadena-Gómez and Nicholson (1987), who observed peroxidase activity 12 h after inoculation of corn plants with *Helminthosporium maydis* or *Colletotrichum graminicola*, so that the highest records of activity of this enzyme were observed 36 h.

After inoculation as they also reported peroxidase activity in non-inoculated plants. Polyphenoloxidase activity started 24 h after application of the treatments, with the highest enzyme activity being reached at 72 h at a concentration of 1.5%, similar results were found by Cadena-Gómez and Nicholson (1987).

Those who found phenols in corn plants 12 h after being inoculated with *Helminthosporium maydis* or *Colletotrichum graminicola*. As for the β-1.3 glucanase enzyme, its activity started 24 h after the application of the treatments and the highest peak of activity was recorded at 120 h, with doses of 1 and 1.5% of Microgeo®.

Conclusions

According to the results obtained in this research, it is concluded that the Microgeo® biofertilizer presents positive results for the induction of enzymatic activity in pyre maize plants, since it induces the activation of enzymes related to pathogenesis (catalase, peroxidase, polyphenoloxidase and β-1.3 glucanase) so that the highest activity occurs 72 h after the application of Microgeo® and in the concentration of 1%.

Cited literature

Angelova, Z.; Georgiev, S. and Roos, W. 2006. Elicitation of plants. Biotechnol. Biotechnological Equipment. 20(2):72-83. https://doi.org/10.1080/13102818.2006.10817345.

Barros, F. C.; Sagata, E.; Ferreira, L. C. and. Juliatti, F. C. 2010 Indução de resistência em plantas à fitopatógenos. Induction of resistance in plants against phytopathogens. Bios. J. 26(2):231-239.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochem. 7(2):248-254. https://doi.org/10.1016/0003-2697 (76)90527-3.

Cadena-Gómez, G. and Nicholson, R. L. 1987. Papilla formation and associated peroxidase activity: A non-specific response to attempted fungal penetration of maize. Physiol. Mol. Plant Pathol. 31(1):51-67. https://doi.org/10.1016/0885-5765(87)90006-3.

Casela, C. R.; Ferreira, A. S. and Pinto, N. F. J. A. 2006. *Doenças na Cultura do Milho*. Embrapa Milho e Sorgo. Circular Técnica. 83(1):1-14.

Colares, M. R. N. and Bonaldo, S. M. 2014. Uso de biofertilizantes na indução de resistência em plantas a patógenos. *In*: Schwan-Estrada, K. R. F.; Silva, C. M.; Maia, A. J.; Faria, C. M. D. R. and Colella, J. C. T. (Org.). Indução de resistência em plantas a patógenos. VII Ed.maringá: suprema gráfica e editora LTDA. 55-72 pp.

Costa, D. F.; Vieira, B. F.; Lopes, E. A. and. Moreira, L. C. B. 2012. Aplicação de fungicidas no controle de doenças foliares na cultura do milho. Ver. Brasileira de Milho e Sorgo, 11(1):98-105.

Cota, L.V.; Costa, R.V.; Sabato, E. O. and Silva, D. D. 2013. Histórico e perspectivas das doenças na cultura do milho. Embrapa Milho e Sorgo. Circular Técnica. 19(1):1-7.

Duangmal, K. and Apenten, R. K. O. 1999. A comparative study of polyphenoloxidases from taro (*Colocasia esculenta*) and potato (*Solanum tuberosum* var. *romano*). Food Chem. 64(3):351-359. https://doi.org/10.1016/S0308-8146 (98)00127-7.

Ferreira, D. F. 2014. Sisvar: a guide for its bootstrap procedures in multiple comparisons. Ciência e Agrotecnologia. 38(2):109-112. http://dx.doi.org/10.1590/S1413-70542014000200001.

Ghini, R. and Kimati, H. 2002 Resistência de fungos a fungicidas. Jaguariúna, SP: Embrapa Meio Ambiente. 78 p.

Krzyzaniak, Y.; Trouvelot, S.; Negrel, J.; Cluzet, S.; Valls J.; Richard, T.; Bougaud, A.; Jacquens, L.; Klinguer, A.; Chiltz, A.; Adrian, M. and Héloir, M. 2018. A plant extract acts both as a resistance inducer and an oomycide against grapevine downy mildew. Frontiers in Plant Sci. 9(1):10-85.

Lanubile, A.; Bernardi, J.; Marocco, A.; Logrieco, A. and Paciolla, C. 2012. Differential activation of defense genes and enzymes in maize genotypes with contrasting levels of resistance to Fusarium verticillioides. Environ. Exp. Bot. 78(1):39-46. https://doi.org/10.1016/j.envexpbot.2011.12.006.

Lever, M. 1972. A new reaction for colorimetric determination of carbohydrates. Analytical Biochem. 47(1):273-279. https://doi.org/10.1016/0003-2697(72)90301-6.

Lorenzetti, E.; Stangarlin, J. R.; Kuhn, O. J. and Portz, R. L. 2018. Indução de resistência à Macrophomina phaseolina em soja tratada com extrato de alecrim. Summa Phytopathol. Botucatu. 44(1):45-50.

Lusso, M. F. G. and Pascholati, S. F. 1999. Activity and isoenzymatic pattern of soluble peroxidases in maize tissues after mechanical injury or fungal inoculation. Summa Phytopathol. 25(3):244-249.

Pascholati, S. F. and Dalio, R. J. D. 2018 Fisiología do Parasitismo: Como as plantas se defendem dos patógenos. *In*: manual de fitopatologia: principios y conceptos. Ouro Fino-MG. Agron. Ceres. 573 p.

Roncatto, M. C. and Pascholati, S. F. (1998) Alterações na atividade e no perfil eletroforético da peroxidase em folhas de milho (*Zea mays*) e sorgo (*Sorghum bicolor*) tratadas com levedura (*Saccharomyces cerevisiae*). Scientia Agricola Piracicaba. 55(3):395-402.

Silva, R. F.; Pascholati, S. F. and Bedendo, I. P. 2008. Indução de resistência em plantas de berinjela por Lentinula edodes e Agaricus blazei contra *Ralstonia solanacearum*: aspectos bioquímicos e biomassa vegetal. Summa Phytopathol. 34(2):137-144.

Sytykiewicz, H. 2015 Transcriptional responses of catalase genes in maize seedlings exposed to cereal aphids' herbivory. Biochem. Systematics Ecol. 60(1):131-142. https://doi.org/10.1016/j.bse.2015.04.015.

Vogelsang, R. and Barz, W. 1993. Purification, characterization and differential hormonal regulation of a β-1, 3-glucanase and 2 chitinases from chickpea (*Cicer arietinum* L.). Planta. 189(1):60-69. https://doi.org/10.1007/BF00201344.

Wordell-Filho, J. A.; Ribeiro, L. do P.; Chiaradia, L. A.; Madalóz, J. C. and. Neri, C. N. 2016. pragas e doenças do milho: diagnose, danos e estratégias de manejo. Florianópolis: Epagri. Epagri. Boletim técnico 170. 82 p.