

Lanthanum *in vitro* control of *Alternaria solani* and induction resistance mechanism against blight tomato plant

Lantânio no controle *in vitro* de *Alternaria solani* e indução de mecanismo de resistência contra pinta preta do tomateiro

Antônio Jussê da Silva Solino¹, Juliana Santos Batista Oliveira², Sergio Augusto Cesnik³, Kátia Regina Freitas Schwan-Estrada⁴

¹Professor Doutor at Faculty of Agronomy of University of Rio Verde, Rio Verde, Goiás, Brazil. antoniosolino@unirv.edu.br. ²PhD in Agronomy of postgraduate of University State of Maringá, Maringá, Brazil. julianaglomer@hotmail.com. ³Undergraduate student of the Agronomy course of University center Inga, Maringá, otmair_cesnik@hotmail.com. ⁴Professor PhD at postgraduate of University State of Maringá, Maringá, Brazil. krfsestrada@uem.br.

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ABSTRACT

Rare earth elements have been tested in control of plant diseases. Lanthanum (La) was tested in the control of *Alternaria solani* (*in vitro*) and tomato early blight (*in vivo*) using the concentration 0; 0.1; 0.2; 0.4 and 0.8 g L⁻¹. *In vitro*, the concentration were diluted in V8 culture medium and evaluated for mycelial growth rate index (MGRI) and pathogen sporulation. *In vivo*, 24 hours after the application of concentration was inoculate the pathogen and 24 hours after the inoculation, leaflets were collected for quantification, the specific catalytic activity and guaiacol peroxidase. The severity of tomato early blight were also analyzed. As 0.27 and 0.28 g L⁻¹ reduces 28% and 50% the MGRI and the sporulation, respectively. Peroxidase and catalase activity was increased by 298% and 151% in tomato treated with 0.5 and 0.4 g L⁻¹ de La, respectively. *In vivo* reduces AUDPC 70% when was applied 0.27 g L⁻¹ La. Lanthanum can be used as resistance inducer in controlling tomato early blight.

RESUMO

Elementos terras raras têm sido testados no controle de doenças de plantas. O Lantânio (La) foi testado no controle de *Alternaria solani* (*in vitro*) e da pinta preta do tomateiro (*in vivo*) utilizando as concentrações 0; 0,1; 0,2; 0,4 e 0,8 g L⁻¹. *In vitro* as concentrações foram diluídas em meio de cultura V8 e foi avaliado o índice de velocidade de crescimento micelial (IVCM) e a esporulação do patógeno. *In vivo*, 24h após a aplicação das concentrações em tomateiro realizou-se a inoculação do patógeno e 24 h após foram coletados folíolos para quantificação da atividade específica de catalase e peroxidase do guaiacol. Também foram analisados a área abaixo da curva de progresso da doença (AACPD) pinta preta do tomateiro. As concentrações 0,27 e 0,28 g L⁻¹ de La reduziram 28% e 50% o IVCM e a esporulação, respectivamente. A atividade de peroxidase e catalase foram incrementadas 298% e 151% em tomateiro tratadas com 0,5 e 0,4 g L⁻¹ de La, respectivamente. *In vivo* observou-se redução de 70% da AACPD da pinta preta ao aplicar 0,27 g L⁻¹ de La. O Lantânio pode ser utilizado como indutor de resistência no controle da pinta preta do tomateiro.

INTRODUCTION

Tomato is one of the most cultivated vegetables in the world (MATOS et al., 2012). Brazil is the ninth largest producer worldwide, producing 4,230,150 tons in 2017 (FAOSTAT, 2019). The culture can be affected by several diseases such as late blight, cancer, wilt, early blight and septoria, which makes

it a culture of high economic risk (LOPES; ÁVILA, 2005). The tomato early blight painting, caused by the fungus *Alternaria solani*, promote damage to producers due to its destructive potential, attacking leaves, petioles, stems and fruits (PEREIRA et al., 2013). This disease is characterized by reduced leaf area and plant vigor, consequently affecting productivity, when not properly controlled (LOPES; ÁVILA, 2005).

The management used to control the tomato early blight is related to the select of the place of installation of the crop, conducting proper fertilization, seed treatment and mainly through preventive and curative spraying with fungicides (KUROZAWA; PAVAN, 2005). The inadequate use of pesticides, considering the high number of applications, lack of rotation of active principles and dose higher than recommended on the product label, can induce the selection of resistant pathogens, with cases reported in the phytosanitary management of tomato diseases, other than affect human health, besides that if the grace period determined by the manufacturer is not respected (BETTIOL, 2004; MORANDI; MAFFIA, 2005). Thus, the search for healthier foods with a sustainable production and in accordance with the preservation of the environment, as the alternative methods of disease control in plants has been highlighting in tomato production.

Alternative pesticides are products of biological, organic and/or natural origin used to control pests and diseases. These products can act directly on the pathogen being target or act indirectly, by activating plant resistance to pests and diseases (PENTEADO, 2001). Plant resistance can be induced by abiotic or biotic molecules, denominated of inducing agent, responsible for activating a plant's defense mechanism against pathogens (HAMMERSCHMIDT et al., 2001; CONRATH et al., 2002).

Among activated defense mechanisms are included pathogenesis-related proteins, such as peroxidases and catalases (VELLOSILLO et al., 2010; CAVERZAN et al., 2012). Hydrogen peroxide and other reactive oxygen species are generated in the plant-pathogen interaction. It is the first arsenal used by plant to directly act on the pathogen and or causing apoptosis, programmed death of plant cells to isolate the site of infection, besides signaling that will trigger genes and other plant resistance mechanisms (MEHDY, 1994; WOJTASZEK, 1997).

Rare Earth elements (REE), who belong to group IIB of the periodic table, have been used to increase seed germination, plant hormone activity and crop yield, they are also being tested for pathogen and disease control (TYLER, 2004; YIAJIA et al., 2008; ALGHOOOL et al., 2013). The static and antimicrobial effect of Rare Earth Yttrium, Cerium, Neodymium, Europium, Dysprosium, Erbium and Lutetium was observed in nine species of phytopathogenic microorganisms (TALBURT; JHONSON, 1967).

Considering the need for alternative control methods in tomato disease managements and the characteristics of Rare Earth antimicrobials, the aim of this work was to verify the effect of Lanthanum on *in vitro* control of *Alternaria solani* and *in vivo* of tomato early blight through direct action or plant resistance induction to pathogens.

MATERIAL AND METHODS

The experiments were conducted at the Plant Resistance Induction to Pathogens Laboratory in the Department of Agronomy at the State University of Maringá – UEM, Maringá, Paraná, Brazil. The *Alternaria solani* isolate was supplied by the State University of Maringá, plated in V8 culture medium, and kept in B.O.D at 25 ± 2 °C, 12 hours of light.

The *in vitro* experiment was to evaluate the antifungal

potential against *A. solani* and the *in vivo* for the activation of resistance mechanism for tomato early blight control. In both experiments the concentrations used were: 0; 0.1; 0.2; 0.4 e 0.8 g L⁻¹ Lanthanum (La), in a completely randomized design with five replications.

To obtain concentrations, *in vitro*, La was diluted in culture medium V8. After solidification, a 0.7 cm diameter *A. solani* mycelium disc from pure colonies was transferred to Petri dishes (Ø = 100 mm). Subsequently the plates were kept in B.O.D at 25 ± 2 °C, 12 hours of light. The variables analyzed were mycelial growth and sporulation. The mycelial growth was determined by measuring in two perpendicular directions the size of the fungal colony every 24 hours from the installation of the experiment until one of the treatments occupied $\frac{3}{4}$ of the plate. With the data the mycelial growth rate index was calculated using the formula by Oliveira (1991) (Equation 1).

$$\text{IMGV} = \frac{\sum (D - D_a)}{N} \quad (\text{Eq. 1})$$

Being: IMGV= mycelial growth rate index; D= current average diameter of colony; D_a= average diameter of the previous day colony; N= number of days after subculture.

To evaluate pathogen sporulation, three mycelium discs (0.9 mm) were collected from the colonies grown in the presence of the treatments and placed in 15 ml falcon tubes containing 5 ml of 1% tween + 1% lactophenol solution to stop germination.

For the *in vivo* test, Santa Clara 5800 tomato group was used, sown in 162-cell plastic trays containing organic substrate from bio-stabilized pine bark (Mec Plant®). The transplantation of the pot seedlings, with a capacity of 3.5 liters prepared with soil and sand (3:1), was performed 15 days after seedling emergence. The treatments were sprayed once, with a hand atomizer, to the point of dripping on the aerial part of tomato plants, when they had four true leaves. Twenty-four hours after the application of the treatments, a leaflet was taken from the second true leaf to measure the enzymatic activity of catalase and peroxidase.

The inoculation was sprayed using a concentration of 4.4×10^5 conidia mL⁻¹ suspended in potato and dextrose (BD) liquid medium, 24 hours after treatment application. The inoculated plants were placed in an intermittent humid chamber for 12 hours.

The variables evaluated were tomato early blight severity and biochemical responses (specific catalase and peroxidase activity). The early blight severity was assessed 20 days after pathogen inoculation, with severity scores on the first leaf using the diagrammatic scale by Boff (1988).

For biochemical analyzes, second leaf leaflets were collected 24h after pathogen inoculation, weighing and rapidly cooling. Subsequently, the leaflets were ground in a mortar using liquid nitrogen and homogenized in 4 mL of 50 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM EDTA and 1% polyvinylpyrrolidone (PVP) was added. The enzymatic extract obtained was stored at -80 °C for the determination of total protein and specific activity of catalase and peroxidase.

To total protein quantification, a 2.5 mL solution of Bradford's reagent was added to 25 µl of enzyme extract under

stirring. Then the spectrophotometer was read, with wavelength 595 nm. As reference, 50 μl of distilled water with 2.5 mL of Bradford Reagent was used (BRADFORD, 1976).

The guaiacol peroxidase activity was determined by the direct spectrophotometric method at 470 nm wavelength, adding 0.5 mL of enzyme extract and 2.8 mL of enzyme substrate prepared with 250 μl guaiacol and 306 μl of hydrogen peroxide in 100 mL of 0.01 M phosphate buffer (pH 6.0) maintained at 30 °C (LUSSO; PASCHOLATI, 1999).

The catalase activity was determined, incubating 0.2 mL enzyme extract in 1.0 mL enzyme substrate containing 65 mM hydrogen peroxide in 60 mM potassium phosphate buffer (pH 7.4) at 37 °C for four minutes. Afterwards, 0.2 mL of 40 mM ammonium molybdate was added and hydrogen peroxide consumption was determined by the catalase present in the extract, measured in a spectrophotometer with a wavelength of 405 nm. A blank was prepared for each sample by adding ammonium molybdate to the reaction mixture, omitting the incubation period. The difference between the white absorbance

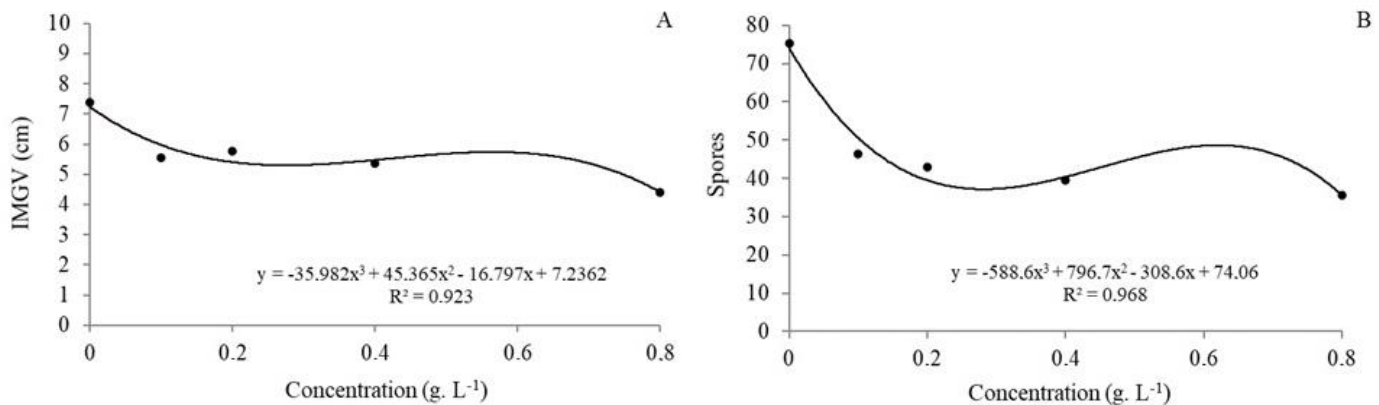
and the incubated sample indicated the amount of hydrogen peroxide used by the enzyme (GÓTH, 1991; TOMÁNKOVÁ et al., 2006)

The *in vitro* and *in vivo* test data were subjected to regression analysis of variance at 5% probability using the statistical program SISVAR 5.6 (FERREIRA, 2011).

RESULTS AND DISCUSSION

The rate of *A. solani* mycelial growth velocity as a function of the concentrations of La was reduced in all treatments, with lower colony growth observed in the concentration of 0.27 g L⁻¹ La, a reduction of 28.16% when compared to the control (Figure 1A). Regarding sporulation of *A. solani*, it was observed that all La concentrations reduced the number of spores, mainly in the concentration of 0.28 g L⁻¹ La (calculated dose), being 50.67% when compared to the control (Figure 1B).

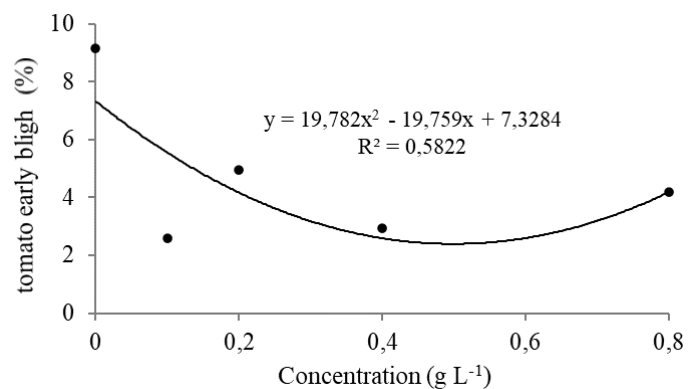
Figure 1. Index of mycelial growth velocity, cv. 15%.6 (A) and number of spores, cv. 16.4% (B) of *Alternaria solani* as a function of diluted Lanthanum concentrations in culture medium (V8).



The *in vitro* tests to control pathogens are fundamental steps to verify the efficiency and effectiveness of pesticides in controlling growth and/or the reproduction factor of phytopathogens. Studying the effect of Lanthanum concentrations on *A. solani* colonies, it was noted a reduction in the growth rate and sporulation of *A. solani*, indicating the fungistatic potential of this rare Earth on this plant pathogen. Lower mycelial growth was also observed when testing Lanthanum in colonies of *Rhizoctonia solani*, *Pythium* sp., *Fusarium solani*, *Sclerotinia sclerotiorum* e *Fusarium oxysporum*, which showed abnormal morphological characteristics such as increased branching, dilation, partial shrinkage and mycelial block, furthermore, the mycelial form irregularly thin tangle (KANGGUO et al., 2006).

The La concentrations also reduced the area below the tomato early blight progress curve. At a concentration of 0.27 g L⁻¹ La, a reduction of 70.25% was observed when compared to the control (Figure 2). However, increasing the concentration to 0.6 g L⁻¹ La reduced the efficiency of early blight control.

Figure 2. Percentage of tomato early blight severity as a function of Lanthanum concentrations, cv. 49.3%.

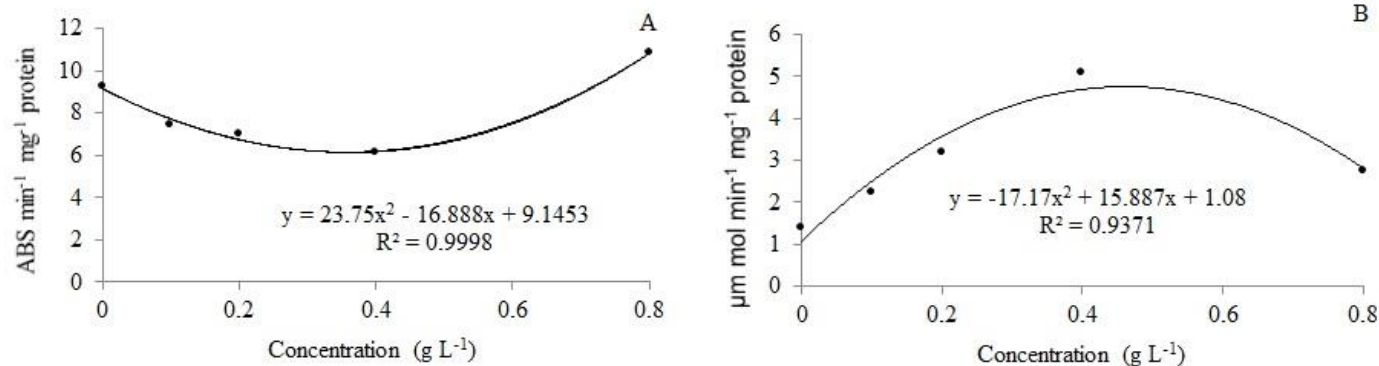


Regarding guaiacol peroxidase activity, it was observed that the application of La at the tested concentrations increased the enzymatic activity. The highest activity was observed when applying the concentration of 0.53 g L⁻¹ La, increasing 298%

when compared to the control (Figure 3A). The catalase activity was increased by 151% when compared to control plants (treated with water) by applying the concentration 0.47 g L⁻¹ La

(Figure 3B). At concentrations higher than 0.47 g L⁻¹ La, a decline in enzyme activity was observed.

Figure 3. Specific activity, cv, 13.4% (A) and catalase (B) guaiacol peroxidase, cv. 31% as a function of tomato Lanthanum concentrations.



When analyzing the severity of tomato early blight, there was a 70% reduction in the area under the disease progress curve (AUDPC) when compared to the control, indicating that this reduction may be correlated to the action of La.

The spraying La on rice leaves reduced the severity of sheath blemishes caused by *Rhizoctonia solani* being the largest control (65%) at the highest concentration tested, 320 mg L⁻¹ seven days after the application of treatments (YAJIA et al., 2008). The reduction in root rot in peanut plants, caused by *Aspergillus niger*, also was observed when applying Lanthanum, Cerium and Neodymium elements. According to the author, these elements reduced the activity of pectinase and cellulase of the pathogen *A. niger* correlating it to the decrease of the fungal infection rate (EMMANUEL, 2013).

The reduction of tomato early blight and increase of guaiacol peroxidase specific activity and tomato catalase when they used biotic elicitors (saprophytic fungi filtrates) (SOLINO et al., 2016). These enzymes mediate the detoxification of the first plant defense event, the oxidative burst, and are involved in curbing infection site formation and severity of tomato early blight disease when triggered by eliciting agent (RESENDE et al., 2009; DUBERY et al., 2012).

The increase in peroxidase and catalase activity observed in Lanthanum-treated tomato leaves is related to the recognition of the chemical element by a plasma membrane receptor present in plant cells (RESENDE et al., 2009; DUBERY et al., 2012). This recognition triggers the initial plant defense process, the oxidative explosion, which refers to the accumulation of reactive oxygen species, which in turn are detoxified by stress-related enzymes such as catalase, guaiacol peroxidase, which were increased by applying it to La (MEHDY, 1994; WOJTASZEK, 1997; RESENDE et al., 2003).

Peroxidase is also involved in a number of essential metabolic processes including lignification and suberization, as well as the synthesis of hydroxyproline-rich glycoproteins and ferulicollated polysaccharides that are involved in the oxidation and polymerization of soluble phenols (BROWNLEADER et al., 1995; SMALLWOOD et al., 1995; HIGARA et al., 2001; RALPH et al., 2004). These enzymes are widely studied because they

effectively participate in the plant defense process, increasing their activity in the presence of infection-causing pathogens and eliciting agents, which is directly related to the reduction of disease severity (KUHNS, 2007). The plant cell walls are the first line of defense against invasion of phytopathogens and peroxidases are key enzymes in the process of their construction (UPADHYAY et al., 2014). This involvement in plant cell wall lignification by elicitor molecule-triggered peroxidases is directly correlated with host resistance during pathogen-host interaction, preventing entry or reducing colonization (AHUJA et al., 2012; UNDERWOOD, 2012).

The La application in cucumber seeds promoted the accumulation of superoxide radicals (reactive oxygen species), which act directly on the pathogen or as a messenger in the activation of defense mechanism (PENGYING; KAOSHAN, 2007). The possible action of these minerals can be mentioned in *Hydrilla verticillata*, where lanthanum and cerium induced increases in H₂O₂ levels, performed in lipid peroxidation, decreased proline, and increased concentration of malonaldehyde, superoxide dismutase and catalase (WANG et al., 2007).

It can not be ruled out that induced resistance mechanisms, such as β -1,3-glucanase and chitinase activity, increased up to three times when treating cucumber cotyledons, resulting in greater resistance of the plant to the attack of pathogens observed in the plant at the same work. The eliciting action of La also described in the increasing the activity of salicylic acid, superoxide radical, β -1,3-glucanase and chitinase in cucumber, acids involved in the defense mechanism activation signaling, demonstrating its potential as a resistance inducer (ZHANG; CHEN, 2007).

CONCLUSIONS

The Lanthanum treatment promoted reduction of mycelial growth speed and sporulation of *Alternaria solani* *in vitro* and reduced the severity of tomato early blight, increasing the activity of peroxidase and catalase in tomato leaflets.

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