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Studying the Morphophysiological and Biochemical Responses of Tomato Treated with Potassium Sorbate Under *Alternaria solani* Stress

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Introduction

Tomato (*Solanum lycopersicum* L.) is the most important fruit or vegetable crop. Its yield and quality are greatly affected by various diseases (Cham et al., 2022). Early blight disease in tomato is caused by *Alternaria solani* (a fungal pathogen) (Saltos-Rezabala et al., 2022). fungicide application is the most common measure used to control its damage. Exploitation of generally regarded as safe (GRAS) and defense inducers have been focused on as alternative strategies to avoid environmental impacts of fungicides (El-Nagar et al., 2020).

Defense inducers enhance plant defense system against stresses through inducing specific changes in plant physiological characteristics. Potassium sorbate (KS) is a safe alternative with antifungal effect in plants that induces Systemic Acquired Resistance (SAR) (Soliman & El-Mohamedy, 2017). The used of potassium sorbate successfully controlled powdery mildew disease in okra with an increase in the activity of peroxidase, chitinase and glucanase enzymes (Soliman & El-Mohamedy, 2017). So, in the present study the effect of potassium sorbate (KS) on physiological, biochemical and defense response of *Alternaria solani*-infected tomato plants was investigated.

Material and Methods

The seeds of tomato of the CH standard variety were prepared. The research greenhouse of Sari University of Agricultural Sciences and Natural Resources was selected as the test site for seed cultivation. Plastic pots with an opening diameter of 17 cm were used for seed cultivation. The culture medium consisted of cocopeat, perlite, and peat moss (MirseyedHosseini et al., 2017). The pots were incubated at 25±2°C, a relative humidity of 75 %, and 16 h light/ 8 h dark photoperiod in growth chamber conditions. From the two-leaf stage until the appearance of the fourth leaf, the seedlings were fed with 100 ml of Spanish Labin fertilizer every twelve days (Boteva, 2016). Plants at the five-leaf stage were treated with potassium sorbate with a concentration of 5 grams per liter (Mirzadeh et al., 2021) and distilled water (as a control). 48 hours later, they were inoculated with *A. solani* (Jabnoun-Khiareddine et al., 2016). Sampling was done at 24, 72 and 168 hours after inoculation.

The fresh and dry weights of the foliar of the plants was measured with a scale. Photosynthetic pigments were undertaken according to Lichtenthaler & Buschmann (2001) method with methanol extract.

0.1 g of leaf tissue was used for measurement of the activity of antioxidant enzymes. The activity of the examined enzymes was done in the following ways: peroxidase enzyme (POD) according to Tang & Newton (2005) method, catalase enzyme (CAT) by the method of Aebi (1984), Superoxide dismutase enzyme (SOD) according to Beauchamp & Fridovich (1971) method, phenylalanine ammonialyase (PAL) according Goldson et al (2008) method with $R^2 = 0.9896$ and $y = 0.1726x - 0.0017$, chitinase enzyme according to Boller et al (1983) method and with $R^2 = 0.9889$ and $y = 0.0214x + 0.001$.

To measure the malondialdehyde (MDA), 0.1 g of leaf sample powdered with liquid nitrogen was homogenized with 1500 microliters of trichloroacetic acid solution (2 %) and according to the method of Ohkawa et al (1979) was investigated.

This research was conducted as a split plot-factorial based on a completely randomized design with three replications. The factors tested included the presence or absence of a pathogen, the use of either potassium sorbate or distilled water (control) for foliar spraying, and three different time courses for sampling (24, 72, and 168 hours). Statistical analysis was performed using SPSS software (version 22). ANOVA and Duncan’s multiple range test (p=0.05) were used to compare the means.

Result and Discussion

Plants treated with inducer in uninoculated conditions showed more fresh and dry weight, which revealed a positive influence of inducer on the growth and development of tomato plants.

Plants activate their defense mechanisms with perception of a pathogen by a plant triggers rapid defense responses via multiple signaling pathways (Manna et al., 2015). One of the primary signals of resistance in diseased plants is the production of reactive oxygen species (ROS), whose excessive increase causes disruption of chlorophyll production and damage to chloroplasts (Ma et al., 1995). The results of this research revealed that photosynthetic pigments decreased significantly in the inoculated plants due to them destruction. The highest amount of chlorophyll a was observed in non-inoculated plants in all three time courses. Plants possess a multifarious ROS defense system comprised of both enzymatic as well as non-enzymatic system to control the level of ROS (Shi et al., 2007). Induction of antioxidant enzymes such as superoxide dismutase, peroxidase and catalase suppress ROS that lead to breakdown hydrogen peroxide (H_2O_2) to oxygen and water (Tanou et al., 2009). The initial and early response in the interaction of the plant with the pathogen is the increase in the POD activity, which is in progress to remove excess amounts of H_2O_2 in the damaged cells. The results of this study indicated that pathogen infection, despite the negative effect on chlorophyll pigment content, improved the physiological condition. The maximum peroxidase amount was observed in plants treated with potassium sorbate in inoculated conditions at 168 hours (25.49 units per gram of fresh weight) that consistent with the study Soliman & El-Mohamedy on okra (2017).

Catalase activity increases dramatically 24 hours post inoculation in the plants treated with potassium sorbate in the non-inoculated condition (26.46 units per gram of fresh weight) that due to potassium sorbate effect in the environment. Which this causes the activation of the defense system and defensive responses at the very beginning of the perception of the stimulus. In a study, the effect of inducer on the early blight disease of tomato has increased catalase enzyme (Khalil et al., 2021).

PAL enzyme is the main and first enzyme of the biosynthesis pathway of phenolic compounds, which increases in biotic and abiotic stresses (Randhir et al., 2006). In this study, PAL enzyme activity showed a significantly increase under the pathogen influence, which is consistent with the results of previous studies (Leul & Zhou, 1999).

The amount of chitinase enzyme activity in plants treated with potassium sorbate in the inoculated condition at 168 hours (1.62 units per gram fresh weight) showed a very significant increase compared to other treatments. Control plants and plants treated with potassium sorbate had the lowest amount of this enzyme at 24 hours post inoculation. It seems that enhancing the activity of this enzyme requires the passage of time and further spread of the disease. In different studies, chitinase enzyme has increased in tomato seedlings under disease stress (Rai et al., 2016; Khalil et al., 2021).

In this study, the amount of MDA in plants treated with potassium sorbate showed a significant decrease in inoculation conditions. This determined that the plants treated with the potassium sorbate inducer had a positive effect on membrane lipid peroxidation when exposed to the pathogen *A. solani*, and these plants were protected against the damage caused by the effects of the fungus.

Conclusions

The results of this experiment showed that potassium sorbate can induce defense mechanisms by triggering induced resistance and improve tomato plant health, additionally, protect the plant against the attacks of *A. solani*. Based on the findings, this inducer can be used to enhance the resistance of the plant against the early blight disease.

Keywords: Defense enzymes, Early blight, Induced resistance, Pathogenic fungus.

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Declaration of conflict of interest

The authors declare that there is no conflict of interest.