Elicitor-Induced Defense Responses in Tomato Plants Against *Xanthomonas gardneri*

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Abstract

Resistance inducers have been focused on promising environment-friendly options to chemical pesticides. In the present study, eleven potential resistance inducers were investigated to evaluate their efficacy in mitigating the adverse impacts of bacterial spot disease in tomato plants. Results revealed that catalase activity and hydrogen peroxide content were sustantially different in inoculated and non-inoculated plants irrespective of the resistance inducer used. The mean concentration of H_2O_2 in the inoculated plants was also increased by 25% compared to the control group. The mean catalase enzyme activity in plants treated by resistance inducers was 0.054 U/mL, while it was 0.111 U/mL in plants solely inoculated with bacteria. The highest SOD activity was recorded in potassium phosphite-treated plants inoculated with the pathogen. Mean catalase activity in inducer-treated plants was 0.052 U/ml, while it was 0.111 U/ml in these plants when inoculated with the pathogen. Malondialdehyde, as a reliable indicator of plant damage upon pathogen attack, exhibited the lowest content in succinic acid-treated plants 8 days after inoculation. This reduction was directly correlated with decreased bacterial spots on the leaves of plants treated with succinic acid. Our results show that potassium phosphite and succinic acid-induced effective defense responses in tomato plants against *X. gardneri* leading to reduced disease severity effect. According to the results, potassium phosphite and succinic acid may be used as potential resistance inducers in tomato plants against *X. gardneri.*

Keywords: Bacterial spot, Catalase, Malondialdehyde, Resistance inducer

Introduction

The growth and development of plants can adversely be affected by several biotic and abiotic stressors (Nawaz et al., 2023). Conversely, as sessile organisms, plants have evolved several strategies to overcome harsh environmental conditions and cope with the different challenges they may face. This adaptation and successful response to these stresses are important for plants to survive and reproduce in an ever-changing climate (Janse van Rensburg et al., 2020)

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Several *Xanthomonas* species are considered the causal agent of bacterial spots in tomatoes. The disease affects all foliar parts of tomato plants, including leaves, stems, and fruits. Several strategies are used to control this disease including exploitation of resistant cultivars, agricultural practices, and bactericide application (Ritchie, 2000). Synthetic bactericides are widely used throughout the world, although their application has been limited owing to resistance development in bacterial populations, as well as, environmental and health concerns (Soto-Caro et al., 2023).

Plants possess multidisciplinary permanent and induced defense systems to deal with stresses. Permanent defense systems include various prefabricated barriers such as polysaccharide-enforced cell walls, as well as, multiple epidermal layers including cuticles and wax. These defensive structures protect the plants from both biotic and abiotic stresses by creating isolating internal tissues (Ullah et al., 2018). Pathogens also have several approaches to defeat plant defense passes through plants' physical barriers and penetrate the internal cells. Pathogen penetration, in turn, activates plant immune responses to inhibit its expansion and further disease proliferation (Hu et al., 2018).

Induced resistance is a plant defense mechanism that triggers physiological, biochemical, molecular, and metabolic changes upon pathogen attack. This particular defense mechanism is highly effective as it is activated solely when required, guaranteeing the most effective allocation of resources. (Jain et al., 2016). Upon recognition of the external stimuli, a multifaceted signaling cascade is triggered and subsequently, several defense responses are activated. Several external stimuli can trigger defense responses including the molecules of pathogen cell walls, degrading plant cell wall metabolites, and other physical and chemical factors (dos Santos and Franco, 2023). The recognition of inducers is usually accompanied by the rapid and transient development of reactive oxygen species (ROS) in apoplast. These ROS act as both antimicrobial agents and signaling molecules and are crucial for downstream signaling pathways against pest and pathogen attacks. These pathways serve as alarms for plant metabolic responses leading to plant protection and damage limitation (Sahu et al., 2022).

ROS are primarily produced in chloroplasts, mitochondria, and peroxisomes. However, under stress conditions, they can also be generated in other plant cell parts such as the cell membrane, cell wall, endoplasmic reticulum, and apoplast (Hasanuzzaman et al., 2021). Stress-induced signaling pathways will be resulted in the over-production of ROS, which subsequently harm plant cells by disrupting the redox equilibrium. This disruption results in lipid peroxidation and deteriorationgradation of chlorophyll, nucleic acids, and proteins (Hasanuzzaman et al., 2021; Schieber and Chandel, 2014) The harmful impacts of excessive ROS are mitigated by plant antioxidant mechanisms (Mittler, 2002).

High concentration accumulation of ROS in plant tissues is known as oxidative burst (Heller and Tudzynski, 2011). According to Zorbrigen et al. (2010)the oxidative burst in plants may induce a hypersensitive response

(HR), which prevents the spread of pathogens to adjacent tissues. HR can also serve as a crucial signal to activate various pathways that regulate plant defense responses and the synthesis of plant hormones (Beers and McDowell, 2001).

Plants typically enhance their antioxidant capacity in response to stress, enabling them to restore the balance between oxidation and reduction (Sahu et al., 2022). ROS also functions as a signaling molecule that activates multiple pathways to suppress pathogens and induce biochemical and physiological changes. This activation helps in overcoming stress (Atkinson and Urwin, 2012; Lamers et al., 2020).

Reactivated oxygen species (ROS) are believed to have a dual role in plants. They are required for several important signaling reactions. In plants, ROS is found to regulate growth, differentiation, redox surfaces, stress signaling, interplay with other organisms, systemic responses, cellular death, as well as toxic by -by-products of aerobic metabolism. This is because they can cause damage to DNA, lipids and proteins (Mittler, 2017).

Plants have numerous antioxidant systems that protect them from potential cytotoxic effects. Among these systems, antioxidant enzymes play a vital role in inhibiting reactive oxygen species (ROS) The main non-enzymatic antioxidants include ascorbic acid (ASA), glutathione (GSH), phenolic compounds such as flavonoids, alkaloids, non-protein amino acids, and α-tocopherol. On the other hand, the enzymatic antioxidants include peroxidase ascorbate (APX), superoxide dismutase (SOD), glutathione reductase (GR), catalase (CAT), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and peroxidase (POX) (Berwal and Ram, 2018). Among the enzymatic systems, superoxide dismutase (SOD) is considered the first line of defense against the oxidative damage caused by ROS in almost all living cells. Catalase (CAT) also plays a crucial role in various plant physiological reactions throughout both the vegetative and reproductive stages (Zheng Yang et al., 2019; Yu Zhang et al., 2020). The successive induction of these pathways ultimalely leads to expression alteration of defense-related genes, development of phytoalexins, and callose deposition (Forman et al., 2010).

It has been revealed that similar to external stimuli, certain natural and synthetic chemicals, frequently referred to as resistance inducers, can activate the plant's immune system (Rabiei et al., 2022). Resistance inducers enhance the general resistance of plants to biotic and abiotic stresses by activating various defense mechanisms enabling plants to respond more effectively to subsequent and/or concurrent biological stresses.

During the past decades several compounds such as potassium phosphite, chitosan, seaweed extract, and salicylic acid have been commercially used as resistance inducers in modern agriculture, and the research is focused on the identification of new compounds with inducing impact on plant defense systems (Reglinski et al., 2023). Potassium phosphite is known to induce defense responses against potato late blight, passion fruit scab, coffee rust, and downy mildew of cucumber (Bonfim et al., 2023; de Fatima Pereira Silva et al., 2023; Liljeroth et al.,

2016; Ramezani et al., 2017). It has been used as fertilizer and fungicide worldwide for several decades.

Azelaic acid, a dicarboxylic acid that naturally occurs in many organisms, has been observed to confer resistance against *Pseudomonas syringae* in Arabidopsis plants when applied to the roots, leading to systemic immunity in aerial tissues (Cecchini et al., 2019).

The current study investigates the efficiency of some potential plant resistance inducers in terms of inhibiting bacterial spot development in tomato plants owing to X. gardneri infection. Different physio-biochemical responses of pathogen-infected tomato plants, as well as, controls were assessed. The results of this study probably aid in understanding the exact defense response of tomato plants treated with each resistance inducer which paves the way for their practical applications for bacterial spot disease control.

Material and methods

Plant growth and treatment

The seeds of the tomato cultivar called CH, were prepared from the company of Falat (Falat Co., Iran). These seeds were sterilized using 1 % sodium hypochlorite solution, followed by rinsing with distilled water. The pots used in the experiment were 21 cm in diameter and contained a combination of peat moss, cocopeat, and perlite in a ratio of 1:2:1. The plants were grown in a greenhouse with controlled conditions with a photoperiod of 12 hours (light/dark), 70% humidity, temperature range of 24-27°C (day/night), and fertilized weekly using the half-strength Hoagland solution (Hoagland and Arnon, 1950). The leaves were sprayed with different resistance inducers (Table 1) when reached to true-4 leave stage.

Pathogen culture and plant inoculation

The bacterium *Xanthomonas gardneri* (IBSF 1782 from the Culture Collection of the Instituto Biológico in Campinas, Bra-

Table 1. List of the potential resistance inductors used to induce resistance against *Xanthomonas gardneri* in tomato plants

Inducer	abbreviation
Potassium phosphite	Kphi
Succinic acid	Suc
Sodium sulfite	NS
Methanol	Met
Beta-aminobutyric acid	BABA
Potassium phosphite and Azelaic acid	Kphi + Az
Potassium phosphite and Azelaic acid and chitosan	Kphi + Az + chit
Potassium phosphite, Azelaic acid, and Methanol	Kphi + Az + Met
Potassium phosphite, Azelaic acid and Salicylic acid	Kphi + Az + SA
Potassium phosphite, Azelaic acid and Sodium sulfite	Kphi + Az + NS
Potassium phosphite and Beta-aminobutyric acid	Kphi + BABA
Control	CTR

zil) was prepared at the Department of Plant Protection, Sari Agriculture and Natural Resources University. The bacteria were grown on NAS medium at a temperature of 27 °C, and then it was suspended in sterile distilled water. The suspension concentration was adjusted to an optical density of 0.05 at 600 ηm, which is approximately 7×106 colony-forming units per milliliter (CFU/mL). Two days after treating tomato plants with inducers they were inoculated with bacterial suspension and the non-inoculated plants were utilized as controls. The plants were kept in the greenhouse environment, under the the same conditions as described previously. By collecting the samples at 2, 4, and 8 days after inoculation and induction, the experiment aimed to analyze the changes in various parameters over time and gain insights into the plant's response to the treatments and pathogen infection.

PAL activity assay

PAL enzyme activity was measured by determinig the quantity of cinnamic acid produced during the reaction between enzyme extract and phenylalanine used to (Barnaby et al., 2008). In each reaction, 0.1 mL of enzyme extract, 500 µL of 500 mM Tris-HCl (pH 7), and 60 µL of 10 mM phenylalanine were combined and incubated at 37°C for one hour. To stop the reaction, 250 microliters of 4 N HCl were used, followed by the addition of one milliliter of toluene to the mixture. After vortexing and a short centrifugation (1000 g for 2 minutes), the absorbance of the upper layer was measured at 290 ηm. The enzyme activity was estimated using a cinnamic acid standard (0 to 15 µg/ml) with an R2 value of 0.987.

Catalase activity assay

A mixture of 25 µL of enzyme extract and a 3 mL of a phosphate buffer (50 mM) containing H_2O_2 (5 mM) was prepared. A spectrophotometer (Aebi, 1984) was used to measure the changes in absorbance at wavelength of 240 ηm..

SOD activity assay

To coduct the SOD activity assay, a mixture of 1000 µL of 50 mM phosphate buffer, containing 1.5 mM EDTA, 10 mM methionine, and 75 µM nitrotetrazolium chloride were prepared. Additionalyy, 100 µL of 1 µM riboflavin and 100 µL of enzyme extract were added to the mixture. The test tubes were then incubated for 10 minutes under a15-watt fluorescent lampplaced 35 cm above them. After turning off the lamp, the absorption changes of the reaction mixture were measured using a spectrophotometer apparatus at wavelength of 560 ηm (Beyer and Fridovich, 1987).

MDA content analysis

An analysis of MDA content was conducted to assess the peroxidation of membrane lipids. The concentration of malondialdehyde, a byproduct of unsaturated fatty acid peroxidation, was determined using the metod outlined by Ohkawa et al. (1979) . Frozen plant tissue (leaf) weighting 0.2 g was homogenized with 5 mL of 0.1% trichloroacetic acid (TCA)and then centrifuged at 10,000 g for 5 minutes. Following this, a mixture of 4 mL of a 20% TCA solution with 0.5% thiobarbituric acid (TBA) was added on 1 mL of the supernatant solution obtained from centrifugation. The resulting solution was heated at 95°C for 30 minutes, cooled on ice and centrifuged again at 10,000 g for 10

minutes. The absorbance of the solution was measured at 532 ηm using a spectrophotometer. with the absorbance of non-specific pigments at 600 ηm subtracted. The. concentration of MDA was calculated using an extinction coefficient of 155 mM-1 cm-1, and the results were expressed in nanomoles per gram of weight.

H_2O_2 accumulation assay

The hydrogen peroxide accumulation was measured using the H_2O_2 accumulation assay as described by Velikova et al. (2000). Plant leaves were gently rubbed in an ice bath containing 0.1% trichloroacetic acid, and the resulting extract was then centrifuged at 10,000 g for 15 minutes using a refrigerated centrifuge. Next, 0.5 mL of the supernatant solution was added to 0.5 mL of a 10 mM potassium phosphate buffer with a pH of 7, along with 1 mL of a 1 M potassium iodide solution. The absorbance was measured at a wavelength of 390 ηm. The amount of hydrogen peroxide in each sample was calculated using a standard curve and reported as micromoles per gram of fresh weight.

Measurement of flavonoids

The total flavonoids content was analyzed using the method described by Chang et al. (2002). To do this, 1 mL of a 2% aluminum chloride solution was added to 1 mL of a 2% methanolic extract and left at room temperature for one hour. Afterward, the absorbance was measured at 420 ηm. Quercetin was used as the standard, ranging from zero to 200 mg (Y = 0.00227X + 0.0175, R2 = 0.97).

Disease severity assay

Assessment of disease severity was conducted following the method of Slopek (1989) with light modifications. Twelve days after inoculating the tomato plants, the disease index (on a scale of 1 to 5) was recorded for each plant based on bacterial spots, and the mean value was calculated as the disease severity. At least 5 infected plants and at least two branches from each plant were selected and photographed using a scanner. The ImageJ software was used to estimate the percentage of symptoms observed on the leaf tissue, using a scale.

where;

1 = no symptoms or spots 2 = 1–25, 3 = 26–50, 4 = 51–75 and 5 = more than 75% disease spots, respectively.

Statistical analysis

All experiments, including plant culture, inoculation, sampling, and measurements were conducted as a split-plot experiment with three replications based on a completely randomized design. The least significant difference (LSD) test was performed at a 1% probability level ($P < 0.01$) for the analysis of mean comparison of growth characters. Data were organized by Excel software and the statistical calculations were performed using SAS 9.1 software.

Results

PAL activity measurement

The findings indicated that the control groups displayed the highest PAL activity on days 4 and 8 following inoculation (Table 2). Succinic acid, on the other hand, demonstrated the lowest PAL activity 2 days post-treatmentand also exhibited the lowest PAL activity inplants infected with pathogens 4 days after inoculation. In contrast, the control plants showed the lowest PAL activ-

ity 2 days after pathogen inoculation, while displayed the highest PAL activity on days following inoculation (Figure 1).

Flavonoids content

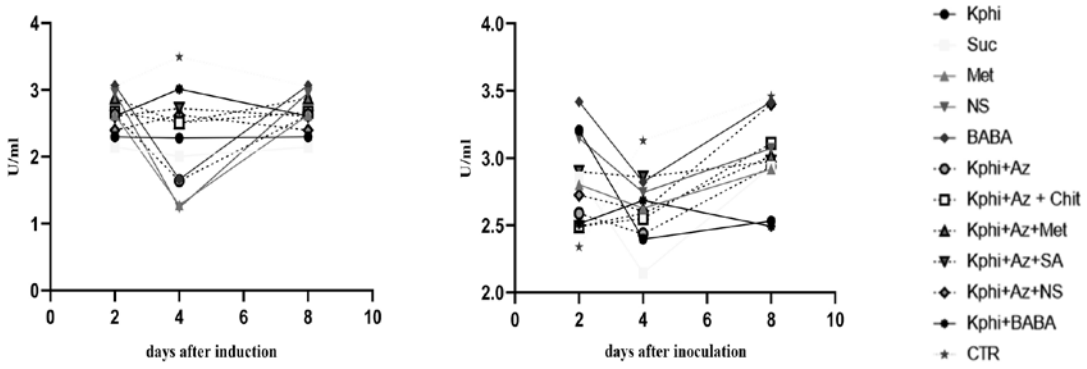
In both experiment conditions, the flavonoid contents observed 8 days post inoculation were higher compared to those observed 2 days after inoculation The average in flavonoid content was measured to be 62.33 mg/f Fw in plants inoculated with the pathogen (Figure 2). Interestingly, this average was found to be 13% higher than the average observed in plants treated solely with the resistance inducers (Table 3). Among the different treatments, the Succinic acid treatment showed the lowest flavonoid concentration after 8 days. However, when plants were incubated with bacteria, this treatment surprisigly showedthe highest flavonoids content. In plants inoculated with the pathogen, flavonoid concentration increased on the 4th days compared to the 2nd days after inoculation. Afterward, the flavonoid content remained relatively constant and did not show any significantly changes on the 8th day after inoculation.

H_2O_2 *accumulation*

The amount of H_2O_2 measured at different time points following bacterial inoculation in the presence of different inducers indicat-

Table 2. Alteration ranges and means of physio-biochemcial characteristics of traits in inducer-treated tomato palnts inoculated or non-inoculated with *Xanthomonas gardneri*

Traits	Average		Maximum		Minimum		Change in
	inoculation	inducer	inoculation	inducer	inoculation	inducer	percentage
PAL (U/ml)	2.76	2.45	4.367	3.275	2.513	2.078	11.25
CAT (U/ml)	0.111	0.052	0.299	0.178	0.009	0.008	53.51
Flavonoid (mg/g FW)	62.33	54.09	71.931	58.620	57.181	51.711	13.23
H_2O_2 (ηmol/g FW)	0.52	0.39	0.634	0.500	0.401	0.235	25.24
MDA (ηmol/g FW)	1.60	1.53	2.258	1.866	1.252	1.196	4.36
SOD (U/mg)	0.30	0.29	0.377	0.449	0.197	0.213	0.04



(**a**) (**b**)

Fig. 1. a) Alterations in PAL activity in tomato plants following the application of a resistance inducer in the absence of pathogen inoculation, b) Variations in PAL concentration in resistance inducer-treated tomato plants infected by *X. gardneri*

ed that succinic acid and BABA treatments exhibited a significant increase compared to other treatments after 2 days after(Figure 3). Moreover, the combination treatment of potassium phosphite+azelaic acid+salicylic acid resulted in the lowest level of H2O2. By the 4th day post-inoculation, after bacterial inoculation, the H_2O_2 levels decreased due to the inducers, only to rise again by the 8th day. The average H_2O_2 concentration in plants treated with inducers was 0.39 ηmol. In plants infected inoculated with the pathogen, there was a 25% increase in , H2O2 levelindicating a significant difference. H2O2 plays a crucial role ingene expression, cell death, and various physiological processes contribute to plant defense machanisms. Particulary in tomato plants, H_2O_2 is essential for combating a variety of disease-causing agents.

SOD activity

In this study, the SOD enzyme's activityshwed anincreaseover time (Figure 4). Notably, a significant difference in average activity was observed between the control group and treatment group after pathogeninoculation . Conversly, there is no significant difference between the treatments in the two

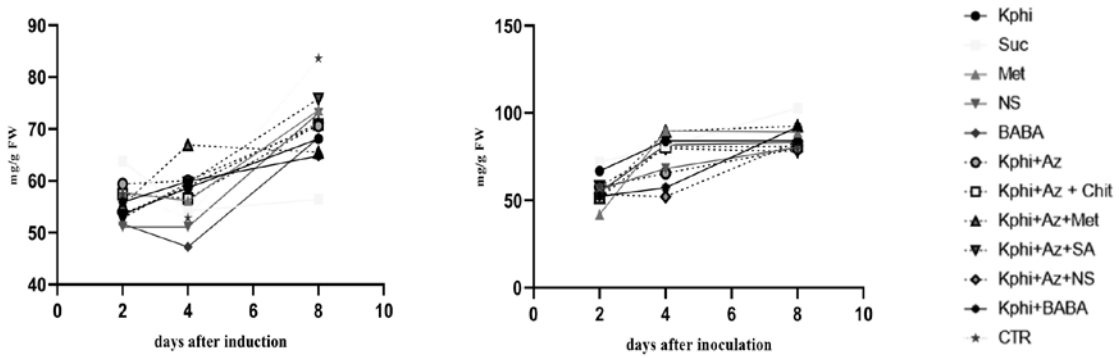


Fig. 2. a). Changes in flavonoid contents in tomato plants treated by various inducers, b) Flavonoid contents alteration in resistance inducer-treated tomato plants infected by *X. gardneri*

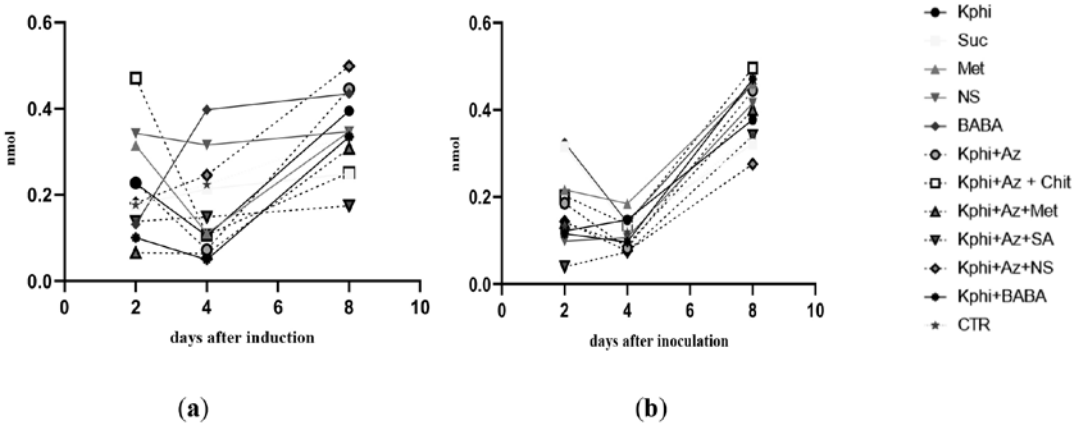


Fig. 3. a) Variations in H_2O_2 content in tomato plants treated by resistance inducers, b) Alterations in H_2O_2 concentration in tomato plants infected by *X. gardneri*

environments (Figure 5). This demonstrates the effect of the inducer on altering the of SOD activity levels, indicating that the inducer led to an increase in SOD levels. As a result, it can be inferred that pathogen inoculation does not influence the rise in SOD activity, suggesting that the increase in SOD levels was primarily due to the effect of inducers. However, there is a difference in the inoculated treatments at different sampling times. The lowest SOD activity level, observed 2 days post-inoculation in the treatment group receiving potassium phosphite+ azelaic acid+ salicylic acid, while hand, the highest SOD activity is linked to the potassium phosphite treatment.

MDA content

In current research, it has been observed that , the activity of the MDA enzyme has gradually increased under conditions of disease stress. This increase was evident in all cases except for t the succinic acid treatment, where the level of MDA showed gradually decreased. Furthermore, the increase in activity was more prominent at 8 days aftert inoculation compared to earlier days. The peak activity was observed at 8 days post inoculation for the combined treatment of potassium phosphite+azelaic acid+salicylic acid, as well as, for the potassium phosphite+

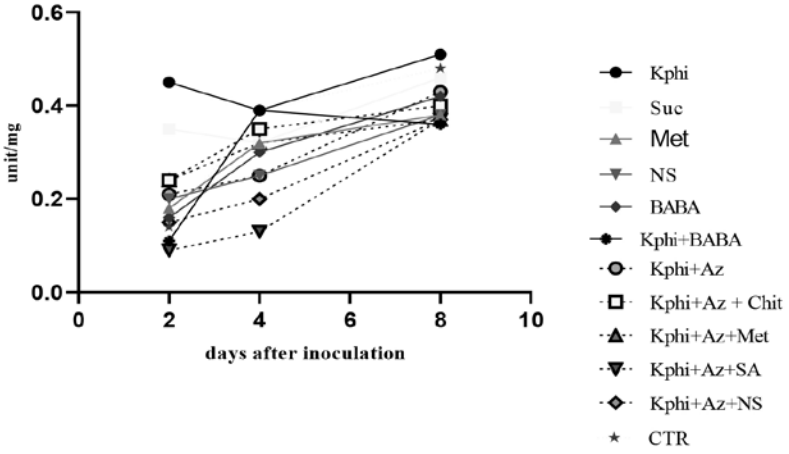


Fig. 4. Variations in SOD activity in tomato plants inoculated by *X.gardneri* bacteria

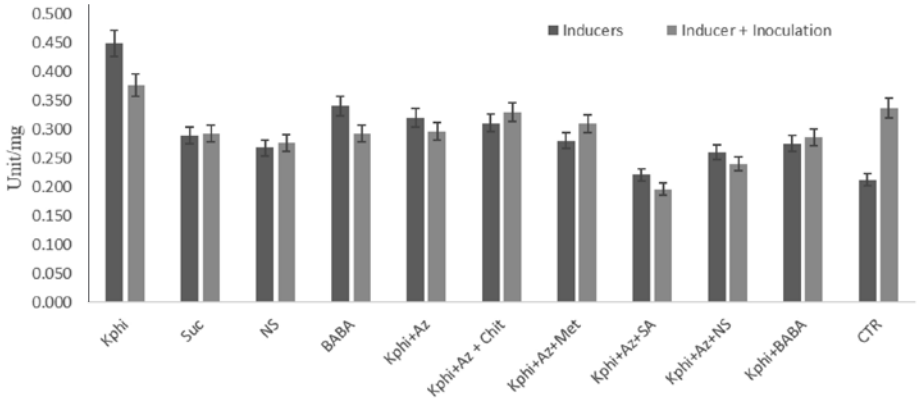


Fig. 5. Mean concentration of SOD enzyme activity in tomato plants treated with resistance inducers and inoculated by *X. gardneri*

azelaic acid+ sodium sulfite treatment (Figure 6). Conversly, the lowest activity was arecorded in the succinic acid treatment.

CAT activity

The mean catalase enzyme activity in plants exposed to resistance inducers was 0.054 U/mL, wherease it measured 0.111 U/mL in plants that were only inoculated with bacteria (Table 3). After Eight days of catalase activity decreased in certain inducers when compared to the control group. Conversly, some treatments showed an increase in catalase activity, with the most significant rise observed in plants treated by BABA. Additionally, across all inducers, catalase enzyme activity was lower in plants inoculated with bacteria than in the control treatment (Figure 7).

Disease severity

According to the results, the plants treated with control and BABA exhibited highest

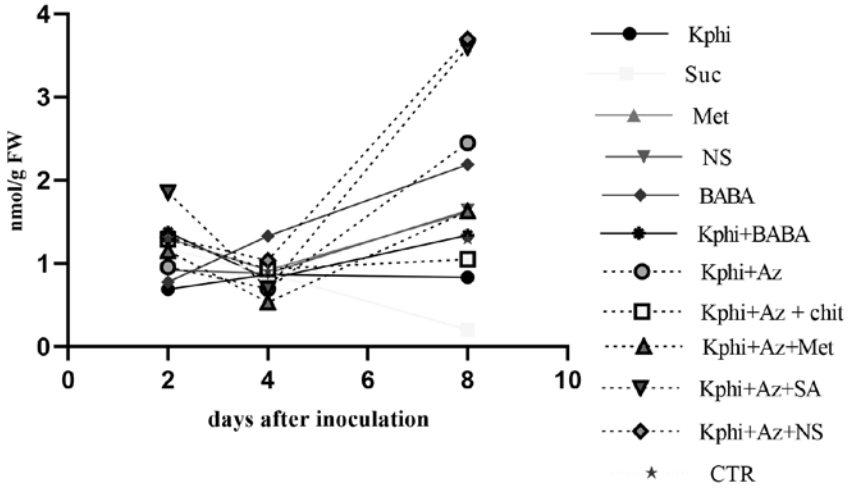
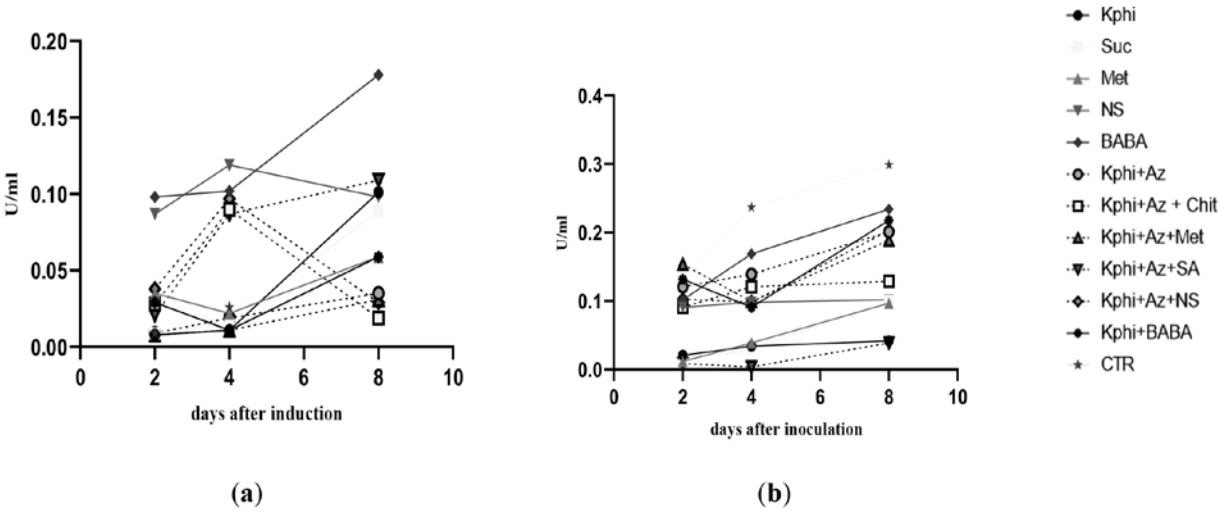


Fig. 6. MDA enzyme activity in resistance inducer-treated tomato plants inoculated by *X. gardneri*



(a) (b)

Fig. 7. a) Alterations in catalase activity in tomato plants treated by resistance inducers, b) Variations in catalase levels in tomato plants inoculated by *X. gardneri*

levels of necrosis on their leaves. Conversly, the plants that were pre-treated with succinic acid + potassium phosphite and then inoculated with bacteria, displayed the lowest number of bacterial sympton spots on their leaves (Figure 8).

Discussion

PAL is actively involved in the synthesis of defense compounds in tomato plants, which are vital for their ability to combat diseases. In a recent investigation conducted by (Debnath et al., 2019), the researchers examined the activity of the PAL enzyme in different tomato genotypes with varying levels of resistance and susceptibility to bacterial wilt. The findings of this study demonstrated that resistant tomato plants exhibited higher PAL activity compared to susceptible plants, indicating a positive correlation between PAL activity and tomato disease resistance. Moreover, a study conducted by Vanitha et al., (2009) revealed that the defense enzymes PAL and PPO play an active role in tomato's plants ability to resist bacterial wilt. In this experiment, Following

Table 3. Mean of physio-biochemcial characteristics inducer-treated tomato palnts inoculated or non-inoculated by *X. gardneri*

	Flavonoid		SOD		H_2O_2		MDA		Catalase		PAL	
Elicitors	Inoculation	inducer	Inoculation	inducer	Inoculation	inducer	Inoculation	inducer	Inoculation	inducer	Inoculation	inducer
Kphi	68.528	53.238	0.377	0.449	0.501	0.378	1.269	1.417	0.032	0.040	2.513	2.290
Suc	71.931	52.338	0.293	0.289	0.401	0.357	1.252	1.196	0.048	0.038	2.542	2.078
Met	73.615	62.512	0.293	0.327	0.288	0.26	1.27	0.93	0.049	0.039	2.78	2.18
NS	59.596	51.711	0.277	0.268	0.502	0.433	1.599	1.584	0.095	0.101	2.682	2.097
BABA	61.740	52.992	0.293	0.341	0.528	0.500	1.873	1.601	0.168	0.126	2.662	2.368
Kphi + Az	58.814	56.869	0.297	0.320	0.555	0.410	1.684	1.253	0.154	0.021	2.546	2.136
Kphi + Az + chit	62.981	54.559	0.330	0.311	0.561	0.427	1.585	1.659	0.114	0.046	2.730	2.590
Kphi + Az + Met	66.083	54.938	0.310	0.280	0.432	0.289	1.268	1.384	0.147	0.017	2.624	2.696
Kphi + Az + SA	61.325	56.685	0.197	0.221	0.413	0.235	2.258	1.316	0.017	0.072	2.879	2.667
Kphi+ Az + NS	57.181	54.207	0.240	0.261	0.419	0.457	2.111	1.615	0.135	0.055	2.759	2.513
Kphi + BABA	61.373	54.416	0.287	0.276	0.558	0.400	1.506	1.866	0.147	0.033	2.638	2.812
CTR	57.548	58.620	0.337	0.213	0.634	0.500	1.586	1.481	0.222	0.031	3.092	3.275
LSD 5%	1.488	1.562	0.932	0.851	0.018	0.021	0.245	0.175	0.908	0.569	0.617	0.186

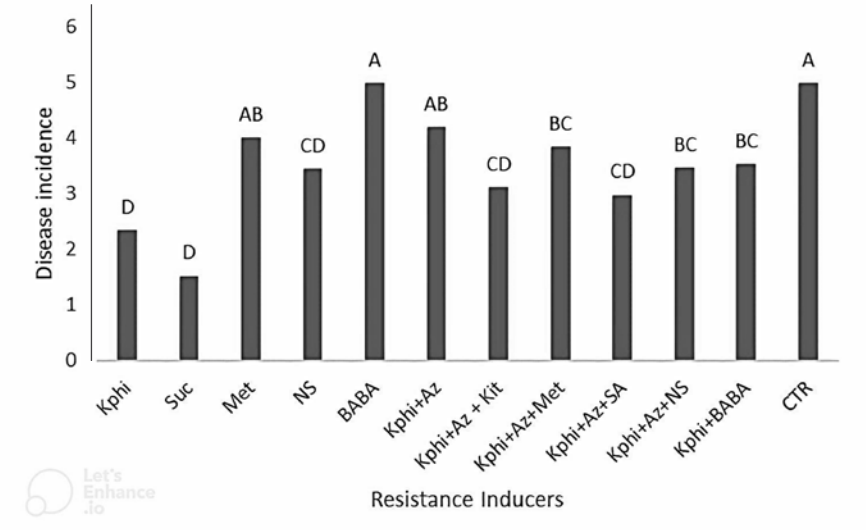


Fig. 8. The disease severity rate in tomato plants treated with a resistance inducer-and inoculated by *X. gardneri*. Leaf necrosis data were collected recorded 12 days after inoculated with *X. gardneri*. The columns represent the mean disease rating on a 1–5 scale method

inoculation, the potassium phosphite treatment showed a higher level of PALactivity compared to the control treatment after two days. This boost in PAL contributed to the plant's defense against the pathogen This enhanced PAL activity is crucial for bolstering the plant's resistance to pathogens. Potassium phosphite is recognized for its ability to promote plant health and vigor by increasing resistance against a variety of plant pathogens, such as oomycetes, soil-borne diseases like Phytophthora and Pythium species, as well aspathogenic bacteria, fungi, and nematodes. Studies have shown that treatments involving potassium phosphite can stimulate the plant immune system, leading to a significant decrease in disease incidence and improving plant resistance to pathogens.

Inducers have been shown to have a significant impact on PAL activity in tomato plants. Studies have demonstrated that these inducers can increase PAL activity, resulting in increased lignin deposition in cell walls, accumulation of phenolics, and enhanced activity of other enzymes, like peroxidase (POD), polyphenol oxidase, and cinnamyl alcohol dehydrogenase (Meena et al., 2022). These changes can contribute to enhancing resistance against pathogens and improve the overall health of the plant. For example, a study showed that water-activated plasma-inducing agents did not directly combat *Xanthomonas vesicatoria*, but bolstered the tomato plant's defenses, enhancing its disease resistance. (Perez et al., 2019). Similarly, the application of silicon in tomato plants has been foumd to elevate POD and PAL activities, along with SA contents, thereby improving disease resistance (Sun et al., 2023). in current study, it was observed that BABA and Kphi treatments increased t PAL levels in tomato plants, indicating a potential impact on the defense responses. The upregulation of PAL indicates that BABA and Kphi treatments could avtivate the plant's defense mechanisms, potentially leading to enhanced resistance against pathogens, pests, or other forms of stress.

Further research is necessary to comprehend the specific mechanisms by which BABA and Kphi treatments induce PAL expression and how this affects plant defense in the long rum. Nonetheless, these findings offer valuable insight into potential strategies for eboosting plant resilience and developing sustainable approaches for plant protection in agriculture.

Flavonoids are secondary metabolites found in plants that play an important role in protecting and defending plants against various biotic and abiotic stresses (Shah and Smith, 2020). Research has shown that flavonoids can induce systemic resistance in tomato plants against bacterial spot, thereby reducing disease severity and limiting the spread of the pathogen (Luiz et al., 2015). In the current experiment, it was observed that succinic acid treatmentresulted in the highest flavonoids levels after bacterial inoculation compared to other treatments. Furthermore, plants treated with succinic acid exhibited the lowest level of disease severity. This indicates that succinic acid not only boosts flavonoid production of flavonoids, which aids in plant defense, but also effectively reduces disease.

The findings from this experiment suggest that succinic acid treatment can effective-

ly enhance the plant's defense responses and provide protection against bacterial infections. By increasing the flavonoids production succinic acid-treated plants exhibit improved antimicrobial and antioxidant properties, contributing to their reduced disease severity.

Furthermore, flavonoids have been linked to reinforcingcell walls and modulating of plant hormone signaling pathways. These effects can assist in controlling pathogens and triggering defense mechanisms. The higher level of flavonoids in succinic acid-treated plants is believed to enhance their capacity-to withstand bacterial infections and exhibit reduced disease symptoms.

Further research is needed to fully comprehend the protective effects of succinic acid on tomato plants. It is essential to elucidate the signaling pathways and gene that are activated by succinic acidto defend the plants. Furthermore, gaining a better understanding of the interaction between succinic acid and the plant's immune system will provide valuable insights into the potential application of succinic acid as a plant defense activator.

Overall, the results highlight the potential of succinic acid as a effective treatment for boosting plant defense against bacterial pathogens. By stimulating flavonoid production and reducing disease severity, succinic acid treatment coul;ld present a sustainable and environmentally approach to disease control in tomato plants and potentially other crops as well.

Hydrogen peroxide (H_2O_2) is a reactive oxygen species (ROS) that plays a crucial role in plant defense mechanisms, particularly in combating bacterial diseases. One of the initial and most effective plant responses to pathogen invasion is referred to as oxidative burst. A high level of ROS, including H_2O_2, is produced in the plasma membrane near the pathogen. Although ROS are naturally produced during normal metabolic processes like photosynthesis and respiration, their levels can rise significantly and temporarily overpower the plant's natural antioxidant defenses. This elevated ROS concentration of can also be toxic to invading pathogens. In this experiment, the average H2O2 level in inoculated plants surpassed that of non-inoculated plants. Additionally succinic acid exhibited the highest H2O2 concentration 2 days after inoculation, indicating its ability to inhibit pathogen growth.

The experiment revealed that the average H2O2 level (hydrogen peroxide) in the inoculated plants was higher compared to non-inoculated plants. This observation suggests that bacterial inoculation triggers an oxidative burst in the plants, leading to an increase in H_2O_2 production. The plant's defense response often involves the generation of reactive oxygen species, including H_2O_2, as a part of the oxidative defense mechanism against pathogens.

Interestingly, the application of succinic acid resulted in the highest concentration of H_2O_2 two days after inoculation. This finding suggests that succinic acid treatment enhances the plant's ability to generate H_2O_2, which in turn inhibits the growth and development of the pathogen. H_2O_2 acts as a signaling molecule and has antimicrobial properties, potentially contributing to the suppression of pathogen growth and spread.

The inhibition of pathogen growth by suc-

cinic acid could be attributed to its ability to activate defense-related mechanisms in the plant. Succinic acid might trigger signal transduction pathways, leading to the activation of defense genes and subsequent production of H2O2. The higher concentration of H2O2 in the succinic acid-treated plants indicates its effectiveness in enhancing the oxidative defense response against the bacterial pathogen.

Overall, the results suggest that succinic acid treatment increases the concentration of H2O2 in tomato plants after bacterial inoculation, suggesting its potential to inhibit pathogen growth. This finding highlights succinic acid as a promising candidate for enhancing plant defense mechanisms and managing bacterial infections in agricultural settings.

Research shows that H_2O_2 plays a critical role in plant defense mechanisms when facing biological stress. Evidence suggests that H2O2 is able to effectively inhibits the proliferation and viability of plant pathogens, thereby limiting the spread of infection (Yergaliyev et al., 2016). In cases of bacterial infection such as *Xanthomonas*, plants produces H_2O_2 as a defense machanism to restrict bacterial growth (Kumar et al., 2011).

Superoxide dismutases (SODs: EC 1.15.1.1) are metalloenzymes found widely in nature and serve as the primary defense agent against reactive oxygen species (ROS). They are also highly efficient components of the antioxidant defense system in plant cells, protecting against the harmful effects of ROS (Berwal and Ram, 2018). Within living cells, SODs facilitate the conversion of superoxide radicals (O_2^-) into hydrogen peroxide (H_2O_2) and oxygen (O_2)playing a vital role in protecting cells from damaging effects generated in various cellular compartments (del Río et al., 2003).

In this study, the treatments led to an increase in SOD levels compared to the control plants. Prticularly, the treatment involving succinic acid and potassium phosphite exhibited the highest SOD levels of 48 hours after inoculation. Several studies have investigated the relationship between SOD activity and Xanthomonas gardneri infection in tomato plants. For instance, a study by Włodarczyk et al., (2023) demonstrated that SOD activity increased in tomato plants treated with nanoparticles combined with conventional fertilizer, resulting in reduced disease severity (Włodarczyk et al., 2023). Another study by Shukla et al., (2018) frevealed that susceptible tomato cultivars responded to nematode infestation by increasing SOD activity, indicating the potential involvement of SOD in plant defense against nematodes. These findings suggest that SOD activity may play an important role in plant defense against *Xanthomonas gardneri* infection.

The experiment showed that the treatments led to an increase in SOD level (superoxide dismutase) compared to the inoculated plants. Superoxide dismutase plays a significant roleas an enzyme in protecting plants from oxidative stress (del Río et al., 2003).

succinic acid and potassium phosphite exhibited the highest significant SOD activity level two days after inoculation. This indicates that these treatments are capable of boosting the plant's antioxidant defense mechanism against *Xanthomonas gardneri*

infection in tomato plants.

Increased SOD activity level triggered by *Xanthomonas gardneri* infection can help mitigate the harmful effects of ROS accumulation. By converting superoxide radicals into H_2O_2 and O_2, SOD aids in preserving redox balance and minimize oxidative damage to plant tissues.

The higher SOD activity observed in plants treated with succinic acid and potassium phosphite-treated plants suggests that these treatments might enhance the plant's ability to scavenge ROS and mitigate oxidative stress caused by Xanthomonas gardneri infection. Consequently, this could contribute to reduced disease severity and improved plant health.

In the current study, the plant treatment with succinic acid resulted in the lowest level of malondialdehyde after inoculation. Additionally, the disease severity index was also low in this treatment, confirming that less damage was inflicted on the plant. . Malondialdehyde is a marker of lipid peroxidation and oxidative stress in plants. The low level of malondialdehyde in the succinic acid-treated plants indicates reduced oxidative damage and stress. This suggests that succinic acid treatment not only reduced oxidative stress but also effectively protected the plants from the harmful effects of the pathogen, resulting in lower disease severity. Researchers studying enzyme activity in tomato plants infected with Septoria lycopersici, foumd that MDA levels of increased significantly with disease severity. Moreover, they observed a simultaneous rise in both MDA and H_2O_2 concentration in other pathosystems, indicating a potential link between oxidative stress and the diseases progression (Silva et al., 2022). Plants produce ROS under abiotic or biotic stress conditions, disrupting the production of biomolecules such as lipids, proteins, and nucleic acids. This disruption leads to an increase in MDA content and plasma membrane permeability, resulting in cell efflux (Zhang et al., 2021). In a study on tomato plants infected with Xanthomonas perforans, an increase in ascorbic acid (ASA) was found to decrease lipid peroxidation and MDA levels. Ultimately, leading to a reduction in disease severity (Alfaro-Quezada et al., 2023).

Catalase plays a role in eliminating reactive oxygen species (ROS) and is a crucial component of plant stress response (Rotich & Mmbaga, 2023). The primary enzyme that responsible for the removal of hydrogen peroxide (H_2O_2) in plants is catalase, predominantly found in peroxisomes/glyoxysomes and in occasionally in mitochondria. The key role of CAT activity in plants is to protectt tissues from damage in the event of a significant rise in H_2O_2 concentration (Zandi and Schnug, 2022).

The average efficiency of plants that were inoculated with bacteria in this research was found to be higher compared to plants that were not inoculated. One potential reason for this enhanced efficiency in tomato plants inoculated with bacteriais the activation of the plants' defense mechanisms. When tomato plants are exposed to pathogens like *X. gardneri*, they might trigger a heightened state of alert, bolstering their overall physiological and immune responses. This activation could improve the plants' efficiency in nutrient uptake, photosynthesis, and resis-

tance to other stressors.

Catalase is also involved in tomato disease resistance, specifically early blight and leaf spot (Shoaib et al., 2019). Studies have demonstrated that tomato plants with enhanced resistance to fungal diseases have higher levels of catalase, indicating its role in mediating disease resistance (Moghaieb et al., 2021). The exact mechanism by which catalase enhances disease resistance in tomatoes is not fully understood, but it is believed to involve the breakdown of H2O2 produced during an attack by pathogens.

Recent research has elucidated the mechanisms by which catalase confers disease resistance in tomatoes. For instance, a study demonstrated that a compound known as alpha-momorcharin (αMMC) can enhance the resistance of tomato plants to Cucumber mosaic virus (CMV) infection. This is achieved by triggering autophagy and suppressing virus replication. Another study revealed that catalase activity plays a role in regulating other enzymes, including peroxidase and phenylalanine ammonia lyase. These enzymes are crucial for plant defense against pathogens (Yang et al., 2023). These findings indicate that catalase plays a mutifaced role in the resistance of tomatoes to diseases, through multiple pathways and mechanisms.

We investigated the efficiency of 11 potential resistance inducers in tomato plants *(Solanum lycopersicum)* challenged by *Xanthomonas gardneri*. The findings revealed that Kphi and Suc were particularly successful in triggering defense responses against *X. gardneri*. The reduced disease incidence in tomato by Khphi and Suc may be a result of increased flavonoids content as well as increased activity of SOD and induction of defense enzymes, while the accumulation of MDA in plant tissues was reduced. Based on the results, Khphi and Suc show promise as potential resistance inducers for protecting tomato plants against the bacterial pathogen *X. gardneri*.

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