CTU Journal of Innovation and Sustainable Development
ISSN 2588-1418 | e-ISSN 2815-6412

DOI:10.22144/ctujoisd.2026.044

# Involvement of induced resistance in the disease-reducing effect of antagonistic *Serratia nematodiphila* CT-78 against rice bacterial leaf blight

Thai Tran Anh Thu, Pham Thiet Trinh, and Nguyen Dac Khoa*

*Institute of Food and Biotechnology, Can Tho University, Viet Nam*

**Corresponding author (ndkhoa@ctu.edu.vn)*

**Article info.**

*Received 15 Apr 2026*
*Revised 22 May 2026*
*Accepted 30 Jun 2026*

**Keywords**

*Bacterial leaf blight, $H_2O_2$, induced resistance, rice, Serratia nematodiphila, Xanthomonas oryzae pv. oryzae*

**ABSTRACT**

*Serratia nematodiphila CT-78 was previously shown to be antagonistic against Xanthomonas oryzae pv. oryzae, thus reducing rice bacterial leaf blight. This study examined the changes in activities of the two defense-related enzymes polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL) to clarify whether induced resistance contributes to the disease-reducing effect of S. nematodiphila CT-78. Applications of $10^7$ CFU/mL CT-78 suspensions by seed soaking, foliar spraying and their combination significantly induced both PPO and PAL activities at early stages which peaked up at 2 days after pathogen inoculation. The combined application of seed soaking and foliar spraying of CT-78 showed stronger and continuous inductions of the enzyme activities 0-7 days after pathogen inoculation. Detection of hydrogen peroxide ($H_2O_2$) accumulation in rice leaf tissues supported the involvement of induced resistance in the observed disease reduction. $H_2O_2$ accumulated in a higher level in leaf tissues of rice plants treated with CT-78 suspensions and inoculated with the pathogen compared to those either treated with CT-78 suspensions or inoculated with the pathogen alone. This indicates enhanced oxidative responses in rice plants against pathogen infection. The results show that the application of S. nematodiphila CT-78 induces resistance at early stages to protect rice plants against bacterial leaf blight.*

## 1. INTRODUCTION

Bacterial leaf blight (BLB), caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most serious bacterial diseases of rice. Severe infections can reduce grain yield by up to 50–80%, threatening food production (Nino-Liu et al., 2006). Current management strategies mainly rely on chemical control and resistant cultivars. However, excessive chemical use can harm the environment, affect human health and promote the emergence of resistant pathogen populations over time (Sundin et al., 2016). The use of antagonistic bacteria is considered a safer and more eco-friendly strategy for disease management. Certain rhizobacteria and endophytic bacteria can suppress plant pathogens directly or indirectly by inducing systemic resistance in host plants (Pieterse et al., 2014). *Serratia nematodiphila* CT-78 has been reported as an antagonistic bacterium against *Xoo*, with antibiosis and induced resistance proposed as key mechanisms involved in disease reduction (Thu & Khoa, 2024). Induced systemic resistance involves the stimulation of plant defense systems, including the accumulation of phenolic compounds and the induction of defense-related enzymes, particularly polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL) (Mandal et al., 2009). PPO

plays a role in the oxidation of phenolics to quinones, which are toxic to pathogens, whereas PAL is a key enzyme in the phenylpropanoid pathway that leads to the synthesis of lignin and phytoalexins (Dixon et al., 2002). In addition, accumulation of reactive oxygen species (ROS), particularly hydrogen peroxide ($H_2O_2$), is an early defense response that helps strengthen the cell wall and acts as a signal to activate the plant defense system (Apel & Hirt, 2004). Therefore, this study aimed to clarify the involvement of induced resistance in the disease-reducing effect of *S. nematodiphila* CT-78 against bacterial leaf blight through (i) changes in the activities of the defense-related enzymes PPO and PAL and (ii) $H_2O_2$ accumulation in rice leaf tissues following bacterial treatment.

## 2. MATERIALS AND METHODS

### 2.1. Materials

The pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), originally isolated from infected rice leaf samples collected in Can Tho City in 2016 and the soil bacterial strain *Serratia nematodiphila* CT-78 were provided by the Plant Pathology Group, Molecular Biology Laboratory, Institute of Food and Biotechnology, Can Tho University.

Seeds of the bacterial-blight-susceptible rice variety Jasmine 85 (Cuu Long Delta Rice Research Institute) (Abebrese et al., 2019) were subjected to the local "three-boil, two-cool" warm-water treatment for 30 min to reduce seed-borne pathogens. Seeds were then soaked in cold water for 24 h and incubated at 28°C for 48 h before sowing.

Alluvial soil was collected from a rice field in Can Tho City.

### 2.2. Methods

The experiment was arranged in a completely randomized design with eight treatments (Table 1) and three replications. Each pot was considered one experimental unit; therefore, each treatment consisted of three pots corresponding to three replications. Rice plants were grown in plastic pots ($30 \times 26 \times 23$ cm$^3$), each containing 7 kg of soil. At each sampling time point, one rice plant was collected from each pot.

#### *2.2.1. Preparation of Serratia nematodiphila CT-78 suspensions*

The bacterial strain *Serratia nematodiphila* CT-78 was cultured for 48 h on Nutrient Agar (NA) medium (Shivaji et al., 2006). Bacterial cells were collected using a round inoculating loop and transferred into 10 mL of sterile distilled water. The suspension was homogenized and adjusted to $10^7$ CFU/mL based on a standard curve established from optical density at 600 nm (Uyen et al., 2018). For seed soaking, rice seeds were immersed in the bacterial suspension for 2 h before sowing. For foliar spraying, 40 mL of the bacterial suspension was applied to each pot using a hand sprayer at 14 days before pathogen inoculation (DBI). The suspension was sprayed evenly to thoroughly wet all leaves of the rice plants in each pot.

**Table 1. Treatments used to investigate induced resistance triggered by antagonistic *Serratia nematodiphila* CT-78 against bacterial leaf blight**

| No. | Treatment | Note |
|---|---|---|
| 1 | Seed soaking with *Serratia nematodiphila* CT-78 suspension ($10^7$ CFU/mL) and pathogen inoculation | Seed soaking + inoculated |
| 2 | Seed soaking with *Serratia nematodiphila* CT-78 suspension ($10^7$ CFU/mL) | Seed soaking + uninoculated |
| 3 | Foliar spraying with *Serratia nematodiphila* CT-78 suspension ($10^7$ CFU/mL) and pathogen inoculation | Foliar spraying + inoculated |
| 4 | Foliar spraying with *Serratia nematodiphila* CT-78 suspension ($10^7$ CFU/mL) | Foliar spraying + uninoculated |
| 5 | Seed soaking and foliar spraying with *Serratia nematodiphila* CT-78 suspension and pathogen inoculation | Seed soaking + foliar spraying + inoculated |
| 6 | Seed soaking and foliar spraying with *Serratia nematodiphila* CT-78 suspension | Seed soaking + foliar spraying + uninoculated |
| 7 | Seed soaking with distilled water and pathogen inoculation | Water + inoculated |
| 8 | Seed soaking with distilled water (control) | Water + uninoculated |

#### 2.2.2. Preparation of *Xanthomonas oryzae pv. oryzae* suspensions

The pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) was cultured for 48 h on Modified Wakimoto medium (Karganilla et al., 1973). Bacterial cells were collected using a round inoculating loop and suspended in 10 mL of sterile distilled water. The suspension was homogenized and adjusted to $10^9$ CFU/mL (Khoa, 2005).

Rice plants were inoculated at 45 days after sowing (DAS) using the leaf-clipping method (Kauffman et al., 1973). Five to ten fully expanded leaves per rice plant were cut 2–3 cm from the tip with scissors previously dipped in the *Xoo* suspension. For the non-inoculated treatments, leaves were clipped in the same way, but the scissors were dipped in sterile distilled water instead of the pathogen suspension.

#### 2.2.3. Collection of leaf samples

Leaf samples were collected daily for seven consecutive days at 24-h intervals from 0 to 7 days after pathogen inoculation (DAI). Samples were collected in the morning; the third leaf from the top was excised at the sheath, wrapped in aluminum foil and rapidly frozen in liquid nitrogen (Xa et al., 2023). The 0 DAI samples were collected immediately after leaf inoculation and used as baseline values to evaluate subsequent changes in enzyme activities.

#### 2.2.4. Extraction of enzymes

Rice leaf samples were collected from three plants, one from each of three pots per treatment. After fine grinding, 0.1 g of leaf tissue was placed in 2 mL Eppendorf tubes containing iron beads. The tubes were immersed in liquid nitrogen for 20 min and subsequently ground into a fine powder using a tissue grinder. The samples were homogenized in 1.5 mL of buffer solution: 0.1 M potassium phosphate buffer (pH 6.5) for polyphenol oxidase and 0.1 M sodium borate buffer (pH 8.7) for phenylalanine ammonia-lyase. The mixtures were centrifuged at 10,000 rpm for 30 min at 4°C. The supernatants were then transferred into 1.5 mL Eppendorf tubes and stored at −20°C for subsequent enzyme activity assays.

*Polyphenol oxidase assays*

Polyphenol oxidase (PPO) activity was assessed by monitoring benzoquinone formation from catechol oxidation through absorbance changes at 490 nm (OD490 nm/min/g leaf tissue), recorded every 30 s for 2 min, following the methods described by Mayer et al. (1965) and Nisha et al. (2012). The experiments were conducted with three replications. The blank sample, set to zero, contained 1.75 mL of 0.1 M catechol and 0.15 mL of 0.1 M potassium phosphate buffer (pH 6.5). The reaction mixture consisted of 1.75 mL of 0.1 M catechol and 0.15 mL of enzyme extract.

*Phenylalanine ammonia-lyase assays*

Phenylalanine ammonia-lyase (PAL) activity was evaluated based on the increase in trans-cinnamate formation from L-phenylalanine deamination, as indicated by changes in absorbance at 290 nm (OD290 nm/min/g leaf tissue) over the reaction period, following the method described by Dickerson et al. (1984). The experiments were conducted with three replications. The blank sample, set to zero, contained 0.7 mL of 0.1 M sodium borate buffer (pH 8.7), 1 mL of 1 M L-phenylalanine, and 0.15 mL of sterile distilled water. The reaction mixture consisted of 0.5 mL of 0.1 M sodium borate buffer (pH 8.7), 1 mL of 1 M L-phenylalanine, 0.15 mL of sterile distilled water, and 0.2 mL of enzyme extract. The reaction was carried out for 40 min at 35°C and terminated by adding 0.2 mL of 5 N HCl.

#### 2.2.5. Detection of hydrogen peroxide

Hydrogen peroxide in rice leaf tissues was detected by 3,3′-diaminobenzidine (DAB) staining following the method described by Daudi and O'Brien (2012). Leaf samples were cut into small pieces and placed in Falcon tubes containing 2 mL of DAB staining solution, adjusted to fully immerse the leaf tissue. As a control, 2 mL of 10 mM $Na_2HPO_4$ was used. Samples were wrapped in aluminum foil to protect them from light exposure, as DAB is light-sensitive, and shaken at 100 rpm for 4 h. The DAB solution was replaced with a clearing solution (ethanol: acetic acid: glycerol = 3:1:1). Samples were incubated in a water bath at 95°C for 20 min to completely bleach chlorophyll, leaving only brown precipitates formed by DAB reacting with $H_2O_2$. The clearing solution was then replaced with fresh solution, and samples were left for 30 min. Samples could be stored at 4°C for up to 4 days before microscopic observation. Finally, samples were examined under a light microscope. The intensity and distribution of brown coloration therefore reflect the level and localization of $H_2O_2$ accumulation in leaf tissues.

### 2.3. Data analyses

The absorbance values obtained from PPO and PAL activity assays were averaged in Microsoft Excel. Differences among treatments within the same

sampling time, as well as changes within each treatment from 0 to 7 days after pathogen inoculation, were analyzed separately using one-way analysis of variance (ANOVA). Mean comparisons were performed using Duncan's multiple range test at the 5% significance level with IBM SPSS Statistics 22.0.

## 3. RESULTS AND DISCUSSION

### 3.1. Results

#### *3.1.1. Polyphenol oxidase activities*

The results demonstrated that PPO activity exhibited dynamic fluctuations depending on both treatment and time after pathogen inoculation (Figure 1).

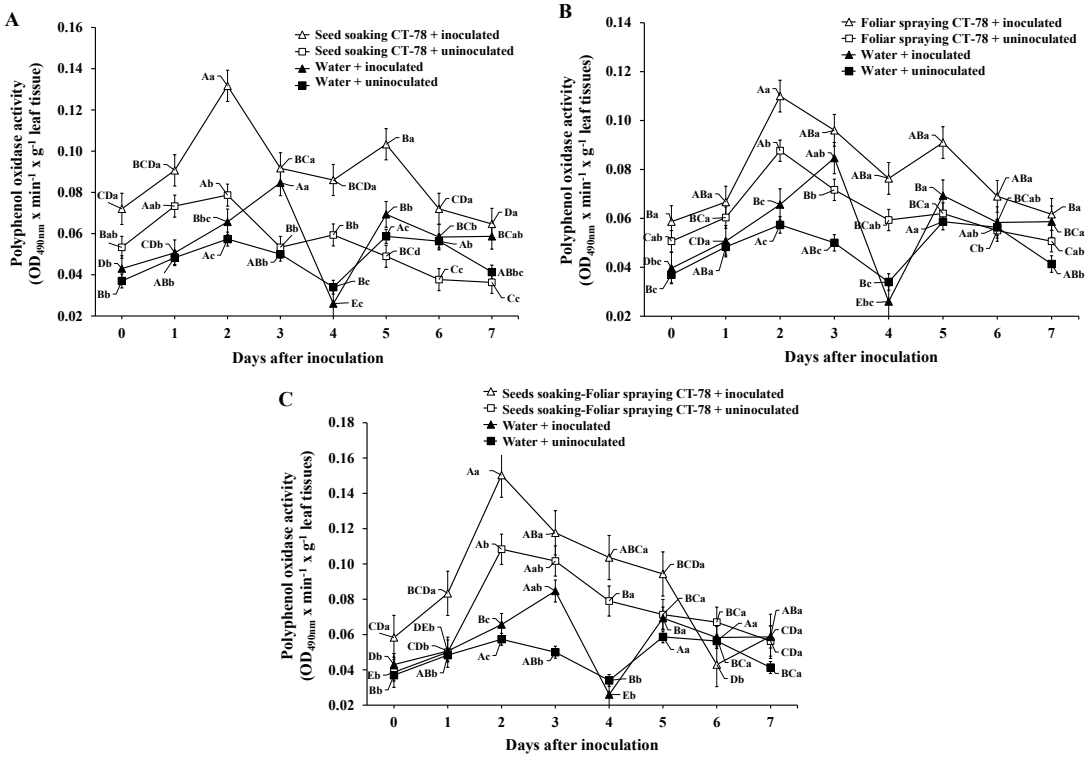


**Figure 1. Changes in the activities of polyphenol oxidase in leaf tissues of Jasmine 85 rice with *Serratia nematodiphila* CT-78 suspensions, evaluated at 24-hour intervals over 7 days after pathogen inoculation**

*(A) Seed soaking; (B) Foliar spraying; (C) Combination of seed soaking and foliar spraying*

*Note: Lowercase letters indicate differences among treatments within the same sampling time, whereas uppercase letters indicate changes within a treatment from 0 to 7 days after inoculation.*
*Different letters indicate statistically significant differences (P≤0.05) by Duncan's multiple range test.*

In the control treatment (water + uninoculated), PPO activity remained low and relatively stable throughout the experimental period, indicating basal enzyme activity under normal physiological conditions. Similarly, across all three application methods, CT-78-treated plants without pathogen inoculation showed only a slight increase in PPO activity, which reached a peak around 2–3 DAI. These results suggest that *Serratia nematodiphila* CT-78 may induce a mild priming effect even in the absence of the pathogen (Figure 1A–C).

In contrast, all inoculated treatments showed increased PPO activity after pathogen challenge. The water + inoculated treatment showed only a moderate increase, with a small peak around 2–3 DAI. However, CT-78-treated and pathogen-inoculated plants showed a stronger response, with PPO activity increasing rapidly to a peak at 2 DAI, then gradually declining at later time points. Among the three application methods, the combination of seed soaking and foliar spraying with CT-78 resulted in the highest PPO activity throughout the

experimental period, indicating the strongest induction of plant defense responses.

Overall, *Serratia nematodiphila* CT-78 effectively induced PPO activity in rice, particularly during the early stages after pathogen inoculation, and the combined application method showed the greatest effect on PPO induction.

### 3.1.2. Phenylalanine ammonia-lyase activities

PAL activity exhibited strong temporal dynamics and clear differences among treatments (Figure 2).

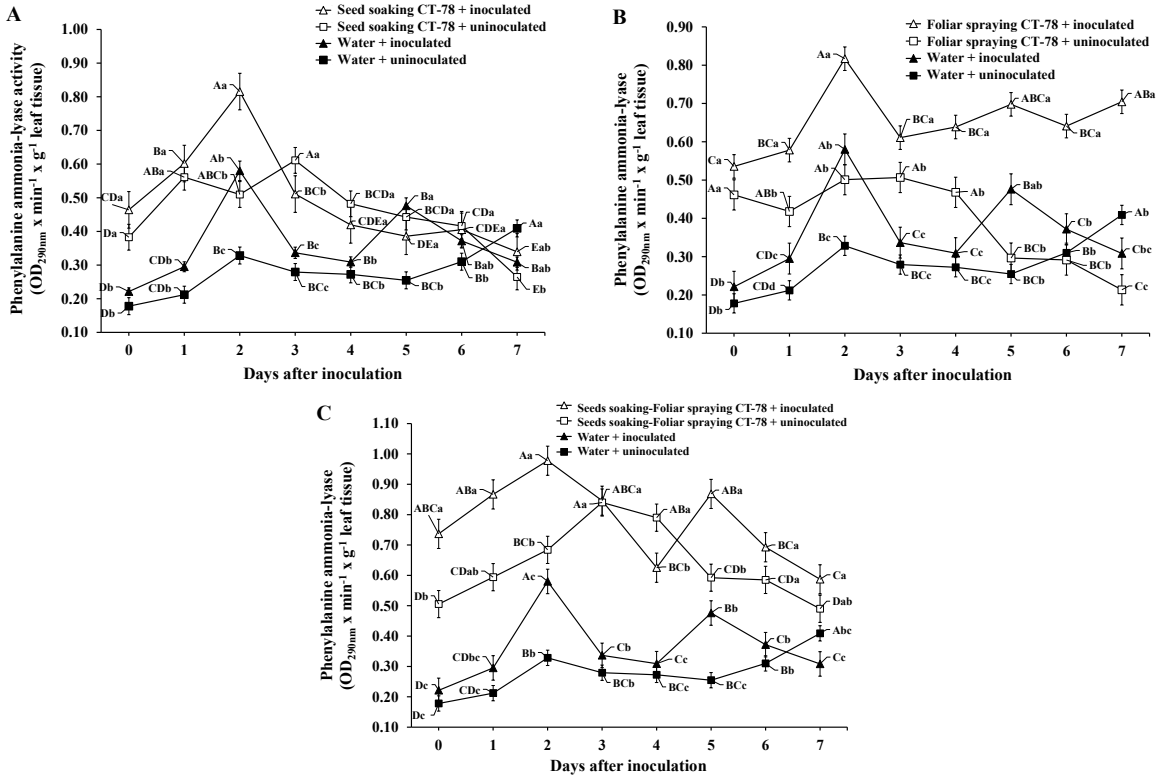


**Figure 2. Changes in the activities of phenylalanine ammonia-lyase in leaf tissues of Jasmine 85 rice with *Serratia nematodiphila* CT-78 suspensions, evaluated at 24-hour intervals over 7 days after pathogen inoculation**

*(A) Seed soaking; (B) Foliar spraying; (C) Combination of seed soaking and foliar spraying*

*Note: Lowercase letters indicate differences among treatments within the same sampling time, whereas uppercase letters indicate changes within a treatment from 0 to 7 days after inoculation.*
*Different letters indicate statistically significant differences (P≤0.05) by Duncan's multiple range test.*

The control treatment (water + uninoculated) maintained the lowest PAL activity throughout the experiment, whereas pathogen inoculation alone (water + inoculated) induced only a moderate increase that reached a peak around 2–3 DAI. In all CT-78-treated plants without pathogen inoculation, PAL activity was slightly higher than that of the control treatment. These results indicate that *S. nematodiphila* CT-78 may stimulate defense responses even in the absence of the pathogen (Figure 2A–C).

In contrast, all CT-78-treated and pathogen-inoculated plants exhibited a rapid and pronounced increase in PAL activity, which peaked at 2 DAI and then gradually declined while remaining higher than that of the control treatment. This pattern suggests early and strong activation of the phenylpropanoid pathway in response to pathogen inoculation. Among the treatments, the combination of seed soaking and foliar spraying with CT-78 resulted in the highest and most sustained PAL activity, indicating the strongest induction of defense responses.

Overall, the results demonstrated that *S. nematodiphila* CT-78 effectively induced rice resistance against bacterial leaf blight through the activation of defense-related enzymes. The combined application method (seed soaking + foliar spraying) provided the most consistent and prolonged activation of PPO and PAL activities.

### *3.1.3. Detection of hydrogen peroxide*

Figure 3 shows rice leaf tissues stained using the 3,3′-diaminobenzidine (DAB) method for the *in situ* detection of hydrogen peroxide ($H_2O_2$). Brown-colored regions observed in the leaf indicated the presence and accumulation of $H_2O_2$. This brown precipitate forms from the reaction between DAB and $H_2O_2$ in the presence of peroxidase enzymes, which produce an insoluble polymer at sites of $H_2O_2$ accumulation.

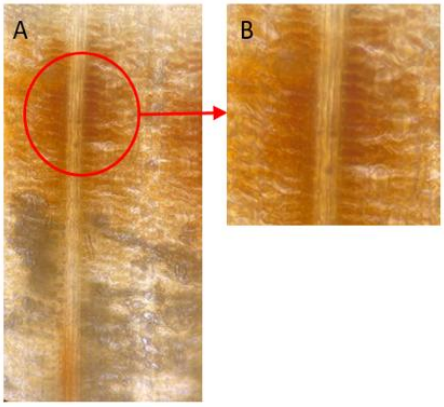


**Figure 3. Detection of hydrogen peroxide ($H_2O_2$) accumulation in rice leaf tissues by DAB staining**

*Note: (A) Rice leaf showing brown staining; the circled area indicates $H_2O_2$ accumulation and (B) magnified view of the selected region. Darker staining represents higher $H_2O_2$ levels.*

Therefore, the intensity and distribution of the brown staining reflect the level and spatial localization of $H_2O_2$ within the leaf tissues. Darker and more intense brown coloration corresponds to higher $H_2O_2$ accumulation, typically associated with stronger oxidative responses during plant–pathogen interactions.

Differences in rice leaf tissues among treatments and observation time points (0–5 days after pathogen inoculation) were observed in the seed soaking treatment with CT-78 suspensions, followed by pathogen inoculation. At 0 DAI, the leaf tissues showed lighter brown staining compared to 1 DAI, when a darker brown coloration was recorded (Figure 4). This indicated that $H_2O_2$ was produced early to inhibit pathogen development and simultaneously functioned as a signaling molecule to activate defense-related enzymes.

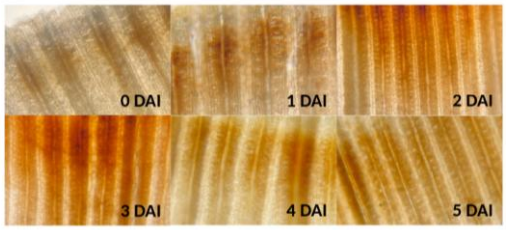


**Figure 4. Accumulation of hydrogen peroxide ($H_2O_2$) in rice leaf tissues at 0–5 days after pathogen inoculation**

*Note: Days after inoculation (DAI).*

Figure 5 shows clear differences in $H_2O_2$ accumulation among the combined seed soaking and foliar spraying treatments at 3 DAI. In the CT-78 + inoculated treatment, strong and widespread brown staining was observed, indicating a high level of $H_2O_2$ accumulation. These findings suggest that the simultaneous presence of the pathogen and antagonistic bacterium induced an oxidative burst in rice leaf tissues. This response is typically associated with induced systemic resistance, in which antagonistic bacteria prime the plant to respond more rapidly and strongly to pathogen attack. In the CT-78 + uninoculated treatment, moderate staining was visible, whereas the water + uninoculated treatment showed weak staining and the water + inoculated treatment showed an intermediate response.

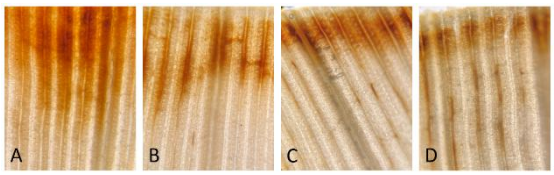


**Figure 5. Accumulation of hydrogen peroxide ($H_2O_2$) in rice leaf tissues at 3 days after pathogen inoculation and *Serratia nematodiphila* CT-78 treatment**

*Note: (A) CT-78 + inoculated, (B) CT-78 + uninoculated, (C) water + inoculated, and (D) water + uninoculated.*

## 3.2. Discussion

This study demonstrated that *Serratia nematodiphila* CT-78 induced the activities of PPO

and PAL in rice plants, particularly during the early stages after pathogen inoculation. These findings are consistent with previous reports showing that beneficial microorganisms can induce systemic resistance in rice and other crops through the activation of defense-related enzymes and oxidative responses. Chithrashree et al. (2011) reported that plant growth-promoting rhizobacteria induced PPO and PAL activities in rice against bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae*. Similarly, Pieterse et al. (2014) described induced systemic resistance as a physiological state characterized by faster activation of defense pathways following pathogen challenge. The induction of defense responses in rice plants was also reported for the rhizobacterial strain *Serratia plymuthica* against the rice blast pathogen *Magnaporthe oryzae* (De Vleesschauwer et al., 2009). El-Esawi et al. (2018) demonstrated that *Serratia liquefaciens* KM4 induced defense responses in maize plants through changes in defense-related enzymes.

PPO is associated with the oxidation of phenolic compounds into quinones, which possess antimicrobial properties and contribute to cell wall reinforcement (Constabel & Barbehenn, 2008), whereas PAL is a key enzyme in the phenylpropanoid pathway involved in the synthesis of lignin, phytoalexins, and other defense-related phenolic compounds (Dixon et al., 2002; Chaman et al., 2003; Sha et al., 2015). The low enzyme activities observed in uninoculated treatments suggested that CT-78 alone did not strongly induce defense responses under normal conditions. However, the slightly higher PPO and PAL activities compared with the control treatment indicate that CT-78 may place rice plants in a primed physiological state. In ISR-associated priming, defense pathways are not fully expressed before infection; instead, plant tissues become more responsive to subsequent pathogen attack (Conrath et al., 2002). Therefore, the strong increases in PPO and PAL activities after pathogen inoculation indicate an enhanced defensive potential in CT-78-treated plants against bacterial leaf blight.

Enzyme activities in the inoculated plants treated with CT-78 showed a rapid and pronounced increase, reaching a peak at 2 DAI, indicating rapid activation of the defensive capacity of rice plants against bacterial leaf blight. Similar results were reported by Chithrashree et al. (2011), in which *Bacillus spp.* strains induced increases in PPO and PAL activities at 48 h after inoculation with *Xanthomonas oryzae* pv. *oryzae*. Bacterial endophytes have also been reported to increase defense-related enzyme activities in tomato plants infected with *Sclerotium rolfsii* (Kumar Sahu et al., 2019).

Among the application methods, the combination of seed soaking and foliar spraying resulted in the highest and most sustained enzyme activities throughout the observation period. This combined effect may promote more efficient colonization of plant tissues and prolonged stimulation of defense signaling pathways, thereby improving induced resistance responses. Seed soaking can stimulate antioxidant enzyme activities and activate physiological defense mechanisms, leading to improved stress tolerance in plants. This process may help establish a primed state, allowing plants to respond more rapidly and effectively to subsequent stress or pathogen attack (Wang et al., 2003). In addition, supplemental application of the antagonistic bacterium CT-78 through foliar spraying 14 days before pathogen inoculation may increase bacterial density on leaf surfaces, thereby enhancing colonization and improving competition with the pathogen during the early stages of infection. Similar results were reported by Truong Van Xa et al. (2023), in which the combined application of seed soaking and foliar spraying with *Kalanchoe pinnata* leaf extract induced enzyme activities and stimulated defense responses in rice plants against pathogen infection. The results further support the role of combined application methods in inducing the plant defense system.

Hydrogen peroxide ($H_2O_2$) is an important reactive oxygen species involved in early plant defense responses. Reactive oxygen species, including $H_2O_2$, superoxide radicals ($O_2^-$), and hydroxyl radicals (·OH), are rapidly generated in plants following biotic stress and function as important signaling molecules in defense activation (Dhiman et al., 2025). However, excessive accumulation of reactive oxygen species can cause oxidative damage to lipids, proteins, and nucleic acids (El-Zohri et al., 2020). Therefore, plants activate antioxidant defense systems and defense-related pathways to reduce oxidative stress and support the biosynthesis of defensive secondary metabolites (Sha et al., 2015). In the present study, the accumulation of $H_2O_2$ after pathogen inoculation suggests an oxidative burst, which is commonly associated with induced resistance. Greater $H_2O_2$ accumulation in CT-78-treated plants indicates that this antagonistic bacterium may enhance the responsiveness of rice

plants to pathogen infection. Meanwhile, the moderate accumulation detected in uninoculated CT-78-treated plants suggests that CT-78 may maintain rice plants in a primed physiological state without causing excessive oxidative stress under normal conditions. These results are consistent with previous studies showing that reactive oxygen species production is an important component of induced resistance during plant–pathogen interactions (Apel & Hirt, 2004). The similar response patterns suggest that CT-78 may activate defense mechanisms commonly associated with rhizobacteria-mediated induced systemic resistance.

The ability of CT-78 to induce defense responses in rice indicates its potential as an environmentally friendly biological control agent against bacterial leaf blight. The combination of seed soaking and foliar spraying showed stronger and more prolonged activation of defense responses, which may improve disease control under net-house conditions. However, several limitations should be considered. The experiments were conducted under controlled conditions, and environmental factors may influence the effectiveness of CT-78 under field conditions. In addition, only PPO, PAL and $H_2O_2$ responses were evaluated in this study, while other defense-related enzymes and signaling pathways may also contribute to induced systemic resistance. Without monitoring CT-78 population dynamics, the relationship among bacterial colonization, enzyme induction, and disease suppression remains indirect. Furthermore, microscopic observation of $H_2O_2$ accumulation was limited to qualitative detection in rice leaf tissues and was not quantitatively analyzed.

Further studies should evaluate other defense-related enzymes, antioxidant systems, and defense gene expression to provide a better understanding of the induced resistance mechanisms triggered by CT-78. Quantitative analysis of $H_2O_2$ accumulation and other reactive oxygen species is also needed to clarify their roles in rice defense responses. In addition, monitoring bacterial population dynamics together with biochemical responses may help explain the relationship among bacterial colonization, enzyme induction, and disease suppression.

## 4. CONCLUSION

This study indicated that *S. nematodiphila* CT-78 treatment activated defense responses associated with induced resistance in rice through increased PPO and PAL activities and elevated $H_2O_2$ accumulation in rice leaf tissues. Applications of CT-78 suspensions significantly increased both PPO and PAL activities, particularly in the early stages after pathogen inoculation, with peak responses at 2 DAI. The combination of seed soaking and foliar spraying with CT-78 resulted in a stronger, more sustained induction of PPO and PAL activities from 0 to 7 DAI. In addition, higher $H_2O_2$ accumulation was observed in rice leaf tissues treated with CT-78 suspensions and inoculated with the pathogen than in those treated with CT-78 suspensions alone or inoculated with the pathogen alone. This indicates an enhanced oxidative response in rice plants against pathogen infection. Overall, the application of *S. nematodiphila* CT-78 induced early systemic resistance responses that protected rice plants against bacterial leaf blight.

## ACKNOWLEDGMENT

This study is funded by Can Tho University under project code T2025-110.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## REFERENCES

K. C., Akromah, R., Gracen V., Offei, S. K., & Danquah, E. Y. (2019). Evaluation of yield, reaction to diseases, and grain physical attributes of some introduced rice hybrids in Ghana. *International Journal of Agronomy*, *2019*(1), 1-8.

Apel, K., & Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annual Review of Plant Biology*, *55*, 373-399. https://doi.org/10.1146/annurev.arplant.55.031903.141701

Chaman, M. E., Copaja, S. V., & Argandoña, V. H. (2003). Relationships between salicylic acid content, phenylalanine ammonia-lyase (PAL) activity, and resistance of barley to aphid infestation. *Journal of Agricultural and Food Chemistry*, *51*, 2227-2231. https://doi.org/10.1021/jf020953b

Chithrashree, A. C. U., Chandra Nayaka, S., Reddy, M. S., & Srinivas, C. (2011). Plant growth-promoting rhizobacteria mediate induced systemic resistance in rice against bacterial leaf blight by *Xanthomonas oryzae* pv. *oryzae*. *Biological Control*, *59*(2), 114-122. https://doi.org/10.1016/j.biocontrol.2011.06.010

Constabel, C. P., & Barbehenn, R. (2008). Defensive roles of polyphenol oxidase in plants. In: A. Schaller (Ed.), *Induced Plant Resistance to Herbivory* (pp. 253-270). Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-8182-8_12

Conrath, U., Pieterse, C. M. J., & Mauch-Mani, B. (2002). Priming in plant-pathogen interactions. *Trends in Plant Science*, *7*, 210-216. https://doi.org/10.1016/S1360-1385(02)02244-6.

Daudi, A., & O'Brien, J. (2012). Detection of hydrogen peroxide by DAB staining in Arabidopsis leaves. *Bio-Protocol*, *2*(18), 1-4. http://www.bio-protocol.org/e263

De Vleesschauwer, D., Chernin, L., & Höfte, M. M. (2009). Differential effectiveness of *Serratia plymuthica* IC1270-induced systemic resistance against hemibiotrophic and necrotrophic leaf pathogens in rice. *BMC Plant Biology*, 9(9), 1-16. https://doi.org/10.1186/1471-2229-9-9

Dhiman, V., Singh, D., Dhiman, V. K., Verma, S. K., & Pandey, H. (2025). *Reactive oxygen species (ROS) and biotic stress: evolving roles under climate change in Climate Change and Biotic Factors* (1st ed.). New York: Apple Academic Press.

Dickerson, D. P., Pascholati, S. F., Hagerman, A. E., Butler, L. G., & Nicholson, R. L. (1984). Phenylalanine ammonia lyase and hydroxy cinnamate: CoA ligase in maize mesocotyls inoculated with Helminthosporium maydis or Helminthosporium carbonum. *Physiological and Molecular Plant Pathology*, *25*(2), 111-123.

Dixon, R. A., Achnine, L., Kota, P., Liu, C. J., Reddy, M. S. S., & Wang, L. (2002). The phenylpropanoid pathway and plant defence - a genomics perspective. *Molecular Plant Pathology*, *3*, 371-390. https://doi.org/10.1046/j.13643703.2002.00131.x

El-Esawi, M. A., Alaraidh, I. A., Alsahli, A. A., Alzahrani, S. M., Ali, H. M., Alayafi, A. A., & Ahmad, M. (2018). *Serratia liquefaciens* KM4 improves salt stress tolerance in maize by regulating redox potential, ion homeostasis, leaf gas exchange and stress-related gene expression. *International Journal of Molecular Sciences*, *19*(11), 3310, 15 pages. https://doi.org/10.3390/ijms19113310

El-Zohri, M., Bafeel, S. O., & Al-Zahrani, W. (2020). Differential oxidative and biochemical responses of tomato and maize leaves to *Spodopter aexigua* herbivory. *Pakistan Journal of Botany*, *52*, 1277-1287. https://doi.org/10.30848/PJB2020-4(23)

Kauffman, H. E., Reddy, A. P. K., Hsieh, S. P. Y., & Nera, S. D. (1973). An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae* pv. *oryzae*. *Plant Disease Reporter*, *57*, 537-543.

Karganilla, A., Natural, M. P., & Ou, S. H. (1973). A comparative study of culture media for *Xanthomonas oryzae*. *Philippine Agriculturist*, *57*, 141-152.

Khoa, N. D. (2005). *Effect of single resistance genes and their pyramid on the diversity of Xanthomonas oryzae pv. oryzae population under field conditions as revealed by insertion sequence-polymerase chain reaction (IR-PCR)* (Master Thesis). University of the Philippines Los Baños.

Kumar Sahu, P., Singh, S., Gupta, A., Singh, U. B., Brahmaprakash, G. P., & Saxena, A. K. (2019). Antagonistic potential of bacterial endophytes and induction of systemic resistance against collar rot pathogen *Sclerotium rolfsii* in tomato, *Biological* Control, *137*, 104014. https://doi.org/10.1016/j.biocontrol.2019.104014

Mandal, S., Mallick, N., & Mitra, A. (2009). Salicylic acid-induced resistance to *Fusarium oxysporum* f. sp. *lycopersici* in tomato. *Plant Physiology and Biochemistry*, *47*, 642-649. https://doi.org/10.1016/j.plaphy.2009.03.001

Mayer, A. M., Harel, E., & Shaul, R. B. (1965). Assay of catechol oxidase: A critical comparison of methods. *Phytochem.*, *5*, 783-789. https://doi.org/10.1016/S00319422(00)836602

Nino-Liu, D. O., Ronald, P. C., & Bogdanove, A. J. (2006). *Xanthomonas oryzae* pathovars: model pathogens of a model crop. *Molecular Plant Pathology*, *7*, 303-324. https://doi.org/10.1111/j.13643703.2006.00344.x

Nisha, S., Revathi, K., Chandrasekaran, R., Kirubakaran, S. A., Narayanan, S. S., Stout, M. J., & Nathan, S. S. (2012). Effect of plant compounds on induced activities of defense-related enzymes and pathogenesis related protein in bacterial blight disease susceptible rice plant. *Physiological and Molecular Plant Pathology*, *80*, 1-9. https://doi.org/10.1016/j.pmpp.2012.07.001

Pieterse, C. M. J., Zamioudis, C., Van der Does, D., & Van Wees, S. C. M. (2014). Signalling networks involved in induced resistance. In D. R. Walters, A. C. Newton, & G. D. Lyon (Eds.), *Induced resistance for plant defense: A sustainable approach to crop protection* (2nd ed., pp. 58-80). John Wiley & Sons.

Sha, P. J., Fan, Y. J., Wang, Z. C., & Shi, X. Y. (2015). Response dynamics of three defense-related enzymes in cotton leaves to the interactive stress of *Helicoverpa armigera* herbivory and omethoate application. *Journal of Integrative Agriculture*, *14*, 355-364. https://doi.org/10.1016/S2095-3119(14)60793-0

Shivaji, S., Chaturvedi, P., Suresh, K., Reddy, G. S. N., Dutt, C. B. S., Wainwright, M., Narlikar J. V., & Bhargava, P. M. (2006). *Bacillus aerius* sp. nov., *Bacillus aerophilus* sp. nov., *Bacillus stratosphericus* sp. nov., and *Bacillus altitudinis* sp. nov., isolated from cryogenic tubes used for collecting air samples from high altitudes. *International Journal of Systematic and Evolutionary Microbiology*, *56*(7), 1465-1473. https://doi.org/10.1099/ijs.0.64029-0

Sundin, G. W., Castiblanco, L. F., Yuan, X., Zeng, Q., & Yang, C. H. (2016). Bacterial disease management: challenges, experience, innovation and future prospects: Challenges in Bacterial Molecular Plant Pathology. *Mol Plant Pathol*., *17*(9), 1506-1518. https://doi.org/10.1111/mpp.12436

Thu, T. T. A., & Khoa, N. D. (2024). Comparison of the disease-reducing effects involving induced resistance against rice bacterial blight of *Serratia nematodiphila* CT-78 and *Kalanchoe pinnata* leaf extracts. *Vietnam Journal of Agriculture and Rural Development*, *Special Issue in Phytopathology (July 2024)*, 96-104 (in Vietnamese).

Wang, H. Y., Chen, C. L., & Sung, J. M. (2003). Both warm water soaking and matriconditioning treatments enhance anti-oxidation of bitter gourd seeds germinated at sub-optimal temperature. *Seed Science and Technology*, *31*(1), 47-56. https://doi.org/10.15258/sst.2003.31.1.06

Xa, T. V., Thoa, T. K., Thu, T. T. A., & Khoa, N. D. (2023). Effects of seed soaking and foliar spraying of *Kalanchoe pinnata* aqueous leaf extracts against rice bacterial leaf blight. *Can Tho University Journal of Innovation and Sustainable Development*, *15*(3), 12-22. https://doi.org/10.22144/ctujoisd.2023.047

Uyen, N. H. N., Van, N. T. C., & Khoa, N. D. (2018). Comparison of the disease-reducing effects of the antagonistic *Bacillus* sp. and *Serratia nematodiphila* against *Xanthomonas oryzae* pv. *oryzae* causing rice bacterial leaf blight. *CTU Journal of Science*, *54*(9B), 59-66 (in Vietnamese). https://doi.org/10.22144/ctu.jvn.2018.181