Biological Forum – An International Journal **14(4): 1342-1349(2022)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Association of some Plant Defense Enzyme activities with Systemic Resistance to Bacterial wilt Disease induced in Tomato Plants by *Pseudomonas aeruginosa* Strain OD13

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(Received 07 August 2022, Accepted 12 October, 2022)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: Controlling *Ralstonia solanacearum* and its impact on tomato crops is a challenge due to its wide host range and environmental adaptability. Chemical pesticides have been used but raise health and environmental concerns. One promising approach is the utilization of antibiotic-producing fluorescent pseudomonads (FPs). FPs are used as a biocontrol tool for bacterial wilt in tomato, enhancing plant growth through induced systemic resistance (ISR). The study aimed to investigate the induction of plant defense-related enzymes against *R. solanacearum* in tomato plants upon treatment with *Pseudomonas aeruginosa* strain OD13. The *Pseudomonas* isolates were obtained from the rhizosphere of solanaceous crops grown in various districts of Odisha. Four treatments were employed, including a control, *R. solanacearum* inoculation, OD13 application, and a combination of OD13 application and *R. solanacearum* inoculation. Tomato seedlings were sampled at various time points, and the activity of stress-related enzymes such as peroxidase (POX), phenylalanine ammonialyase (PAL), polyphenol oxidase (PPO), lipoxygenase (LOX), and total phenol content were measured. The maximum activity of peroxidase was observed in the treatment of *P. aeruginosa* OD13 + *R. solanacearum* after 24 hours of incubation (14.81±0.48 µ.mol/Δ 470 nm/min/mg protein) compared to the control. Similarly, the treatment of *P. aeruginosa* OD13 challenged with R. solanacearum showed higher levels of PAL and PPO activity at 36 hours after inoculation (43.07±0.62 µ.mol/trans cinnamic acid/min/mg protein and 44.33±1.25 Δ A420 nm/min/mg protein, respectively). Lipoxygenase (LOX) activity was higher in *P. aeruginosa* OD13 challenged with *R. solanacearum* at 48 hours (49±1.42 Δ A234 nm/min/mg protein). The total phenol content was significantly increased with *P. aeruginosa* OD13 + *R. solanacearum* (83.5±1.32 µg of catechol/min/mg protein) and *P. aeruginosa OD13* alone (70.5±0.98 µg of catechol/min/mg protein) treated tomato seedlings at 24 hours after inoculation compared to the control. These results suggest that *P. aeruginosa* OD13 induces a defense response and contributes to resistance against bacterial wilt disease in tomato plants. Overall, the findings of this study demonstrate that *P. aeruginosa* strain OD13 can effectively induce plant defense-related enzymes in tomato plants. These induced defense responses have the potential to enhance the plant's resistance against various pathogens, highlighting the potential use of OD13 as a beneficial rhizobacterium for plant protection in tomato cultivation.

Keywords: Pseudomonas, defense-related, peroxidase, phenylalanine ammonialyase, polyphenol oxidase, Lipoxygenase.

INTRODUCTION

Bacterial wilt disease caused by *Ralstonia solanacearum* is a severe threat to tomato (*Solanum lycopersicum* L.) cultivation (Artal *et al.,* 2012, Nion and Toyota 2015), causing yield losses up to 90% in India (Ram *et al*., 2013). The bacterium colonizes the root surface, attacks the plant through the xylem vessel, and inhibits nutrient and water translocation, leading to wilting and death of tomato plants (Murti *et al*., 2021). Chemical control strategies, including antibiotics and copper compounds, have negative effects on the environment, soil fertility, and human health. Biological control, such as beneficial microorganisms, are recommended as more sustainable alternatives. These methods can reduce toxicity and residual effects while effectively controlling the disease.

Using biological control through beneficial microorganisms is a promising and eco-friendly method for managing bacterial wilt disease in tomato crops. Beneficial microorganisms, such as *Pseudomonas spp*., offer a sustainable alternative for controlling bacterial wilt disease in tomato plants. These microorganisms can effectively compete with the pathogenic bacterium, produce antimicrobial compounds and stimulate plant defense mechanisms without causing residual toxicity or harm to the environment.

The bacteria *Pseudomonas*, which is associated with host plants, has been found to enhance the synthesis of defense-related molecules in the plants. Plant cells possess advanced antioxidative pathways that involve the activation and accumulation of common ISR enzymes, such as peroxidase (PO), phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO), lipoxygenase (LOX), chitinase, β-1,3-glucanase, and pathogenesis-related protein (PR). These antioxidant enzymes lead to the thickening of cell walls, structural deformation, deposition of callose at the site of pathogen invasion, and accumulation of phenolic compounds in response to pathogen attack. (Choudhary *et al.,* 2007; Kurabachew and Wydra 2014; Jayapala *et al*., 2019; Bhattacharyya *et al.,* 2020). Considering the information provided, the current study was conducted to examine whether *Pseudomonas aeruginosa* strain OD13 could stimulate the synthesis of defense-related enzymes and confer resistance to bacterial wilt.

MATERIAL AND METHODS

A. Isolation of Bacteria

Soil samples were collected from the rhizosphere of solanaceous crops in various districts of Odisha and isolated on King's B medium agar. Then the isolates were screened against *Rolstonia solanacearum* and the potential strain was identified as *Pseudomonas aeruginosa* strain OD13 (Accession number OQ781265).

B. Green house experiment

The greenhouse experiments were conducted using earthenware pots and followed a complete randomized block design (CRBD) with triplicates. The tomato seedlings were grown in trays filled with sterilized field soil, and Pusa Ruby tomato seeds were sown according to the assigned treatments. After 21 days, the tomato seedlings were transplanted into larger pots to undergo the different treatments.

C. Effect Pseudomonas aeruginosa strain OD13 against R. solanacearum by plant defence enzymes

Pseudomonas aeruginosa OD13 was used to elicit a defensive response against *R. solanacearum*. The experimental treatments consisted of the following treatments: (1) Control (pusa ruby seeds treated with sterile distilled water); (2) Inoculation of *R. solanacearum*; (3) Application of *Pseudomonas aeruginosa* OD13 to seeds and soil (25 mL of bacterial cells at a concentration of 7×10^8 Cfu/ml); (4) Application of *Pseudomonas aeruginosa* OD13 to seeds and soil (25 mL of bacterial cells at a concentration of 7 × 108 Cfu/ml) along with *R. solanacearum*. These treatments were duplicated three times. Tomato seedlings, both inoculated and uninoculated, were harvested at 0, 12, 24, 36, 48, 60, and 72 hours after the challenge inoculation and stored at -20ºC for subsequent analysis. Throughout the three-day study period, samples were collected from all treatments every 12 hours to measure the activity of stress-related enzymes involved in defense mechanisms, including peroxidase (POX), phenylalanine ammonialyase (PAL), polyphenol oxidase (PPO), lipoxygenase (LOX), and total phenol content.

(i) Estimation of peroxidase (POX) activity. Tomato seedlings (1g) were homogenized with 1 ml of 10 mM phosphate buffer (pH 6.0) using liquid nitrogen to create a fine paste. After centrifugation at 12,000 rpm for 20 mins at 4ºC, the supernatant was collected as the enzyme source for peroxidase (POX) analysis. The modified Hammerschmidt *et al.* (1982) POX assay included a 3 ml reaction mixture of 0.25% (v/v) guaiacol in 10 mM potassium phosphate buffer (pH 6.0) with 10 mM hydrogen peroxide. After adding 100 µl of crude enzyme extract, the reaction was initiated and measured at 470 nm for 1 min, with additional readings taken every 30 sec for 3 mins at 420 nm. POX activity was calculated as changes in absorbance min^{-1} mg^{-1} of fresh tissue and compared to the control (Konappa et al., 2016).

(ii) Estimation of phenylalanine ammonialyase (PAL) activity. The PAL (phenylalanine ammonialyase) activity was evaluated following a modified procedure based on Lisker *et al.* (1983). Tomato seedlings (1g) were macerated with 1 ml of 25 mM Tris-HCL buffer (pH 8.8) using liquid nitrogen, yielding a fine paste. After centrifugation at 10,000 rpm for 30 min at 4ºC, the supernatant was utilized for the enzyme assay. The reaction mixture consisted of 1 ml enzyme extract, 0.5 ml of 50 mM L-phenylalanine substrate, and 0.4 ml of 25 mM Tris-HCL buffer (pH 8.8). Following a 2-hour incubation at 40ºC, the reaction was terminated by adding 0.06 ml of 5 N HCl. Absorbance at 290 nm was measured against a blank. The enzyme activity was expressed as mol of trans-cinnamic acid produced per milligram of protein per hour (mol of CA mg^{-1} protein h^{-1}) and compared to the control (Kavitha and Umesha 2008).

(iii) Estimation of polyphenol oxidase (PPO) activity. Polyphenol oxidase activity was determined using the method described by Mayer *et al.* (1965). Tomato seedlings (1g) were homogenized with 10 mM phosphate buffer (pH 6.0) using liquid nitrogen in a pre-chilled mortar and pestle, resulting in a fine paste. After centrifugation at 12,000 rpm for 20 minutes at 4ºC, the supernatant was collected as the enzyme source. The reaction mixture consisted of 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5) and 200 µl of the enzyme

extract. The reaction was initiated by adding 200 µl of 10 mM catechol. The rate of catechol oxidation was measured at 420 nm for 1 minute. The PPO activity was calculated as the change in absorbance per minute per milligram of fresh tissue. The experiments were performed three times and compared to the control, following the protocol of Kavitha and Umesha (2008).

(iv) Estimation of Lipoxygenase (LOX) activity. Lipoxygenase (LOX) activity was assessed following the method outlined by Borthakur *et al.* (1987). Tomato seedlings (1g) were homogenized in 0.2 M sodium phosphate buffer (pH 6.5) using liquid nitrogen in a pre-chilled mortar and pestle, resulting in a fine paste. The reaction mixture consisted of 0.05 ml of the enzyme extract, 2.7 ml of 0.2 M sodium phosphate buffer (pH 6.5), and 0.3 ml of 10 mM linoleic acid in Tween 20. LOX activity was measured by monitoring the appearance of conjugated diene hydroperoxide at 234 nm using a spectrophotometer. The activity of LOX was expressed as the increase in absorbance per minute per milligram of fresh tissue. The experiments were performed three times concurrently with the control, following the methodology described by Kavitha and Umesha (2008).

(v) Estimation of Total Phenol Compounds. To extract the compounds from tomato seedlings, one gram of the seedlings was homogenized using a mortar and pestle to obtain a fine paste. The homogenate was then extracted with 10 ml of 80% methanol at 70ºC for 15 minutes. After extraction, the mixture was centrifuged at 12,000 rpm for 20 minutes at 4ºC.For the analysis, 1 ml of the methanolic extract was combined with 5 ml of sterile distilled water and 250 µl of Folin Ciocalteau reagent (1 N). The resulting solution was incubated at 25ºC for 30 minutes. Upon incubation, the tube exhibited a blue color, and the absorbance was measured at 725 nm using a spectrophotometer. The activity was expressed as the increase in absorbance per microgram of catechol per milligram of fresh tissue. The experiments were conducted three times and compared to the control, as described by Konappa *et al*. (2016).

RESULT AND DISCUSSION

Defence enzymes – Induced Systemic Resistance (ISR) activities

The enzyme activity assays for Induced Systemic Resistance (ISR) were conducted on different treatments involving pathogen-infected plants, including *Pseudomonas aeruginosa* OD13, as well as uninoculated control plants. In comparison to other treated seedlings, both the pathogen-infected and *P. aeruginosa*-challenged tomato seedlings exhibited higher enzyme activity for all the enzymes tested.

Our research demonstrates that the *Pseudomonas aeruginosa* OD13 isolate acts as a potent antagonist against the wilt pathogen *R. solanacearum* by inducing the activity of several ISR elicitor molecules, including POX, PPO, PAL, LOX, and total phenol content. These findings align with previous studies conducted by Vanitha and Umesha (2011); Nithya *et al*. (2019), which showed that *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* VMKU2 induce plant defense mechanisms like ISR to effectively control wilt disease in tomatoes and sheath blight in rice. Our study further revealed that the expression of all five defense molecules increased in tomato plants induced by our OD13 isolate and challenged with *R. solanacearum*.

A. Estimation of POX

The activity of POX (peroxidase) enzyme increased after microbial interactions and reached its maximum level at 24 hours after inoculation. Tomato seedlings challenged with *R. solanacearum* exhibited lower POX activity (14.81±0.48 µ.mol/Δ 470 nm/min/mg protein) compared to tomato seedlings treated with *P. aeruginosa* OD13. In contrast, tomato seedlings treated with *P. aeruginosa* OD13 alone showed higher POX activity (15±0.37 µ.mol/Δ 470 nm/min/mg protein) than the untreated control. However, the highest POX activity (24.2±0.68 µ.mol/Δ 470 nm/min/mg protein) was observed in *P. aeruginosa* OD13 treated tomato seedlings that were also challenged with *R. solanacearum*, surpassing the activity in the control.

Peroxidase (POX) plays a crucial role in lignin biosynthesis, providing support to plant tissues and preventing the entry of pathogens. However, high POX activity can lead to oxidative stress and damage macromolecules (Ramamoorthy *et al.*, 2002; Nithya *et al*., 2019). In our study, we observed increased peroxidase activity at 24 hours post-inoculation in tomato seedlings treated with *P. aeruginosa* OD13 and challenged with *R. solanacearum*. The interaction of pathogens and antagonistic microbes can elevate the levels of peroxidases in plant cell walls. These findings align with previous studies by Konappa *et al*. (2016), which showed increased POX activity in tomato seedlings treated with lactic acid bacteria against *R. solanacearum* at 24 hours. Similarly, Vanitha and Umesha (2011) reported higher peroxidase activity in tomato seedlings treated with *Pseudomonas fluorescens* DABBV4 against *R. solanacearum* at 24 hours. Xie *et al*. (2017) demonstrated elevated POX activity in patchouli plants inoculated with *R. solanacearum* at 72 hours, induced by *Pseudomonas fluorescens* and *Pseudomonas sp*. VSMKU2. Increased POX activity has also been observed in various plants such as cucumber (Chen *et al.*, 2000), rice (Nandakumar *et al.*, 2001; Nithya *et al.*, 2019), and tomato (Ramamoorthy *et al*., 2002) under the influence of different microbes. The heightened POX activity in intercellular spaces promotes cell wall stiffening, potentially reducing cell growth under stress conditions and serving as a mechanical adaptation to adverse environments.

B. Estimation of PAL

PAL activity was assessed in tomato seedlings treated with *Pseudomonas aeruginosa* OD13 and challenged

with *R. solanacearum* and the highest PAL activity (43.07±0.62 µ.mol/trans cinnamic acid/min/mg protein) was recorded at 36 hours after inoculation. Tomato seedlings treated with *P. aeruginosa* OD13 alone exhibited higher PAL activity (34.03±0.74 µ.mol/trans cinnamic acid/min/mg protein) compared to tomato seedlings challenged with *R. solanacearum* (31.1±0.78 µ.mol/trans cinnamic acid/min/mg protein).

The results of the current study indicate that PAL activity was increased in tomato seedlings treated with *P. aeruginosa* OD13, which helps prevent the entry and colonization of *R. solanacearum*. PAL, being involved in the biosynthesis of lignin, accumulates and strengthens the defense mechanism against pathogen infection. The study showed that PAL activity was significantly higher in *P. aeruginosa* OD13-treated tomato seedlings challenged with the pathogen, peaking at 36 hours after inoculation. Comparatively, tomato seedlings treated with P. aeruginosa OD13 alone also exhibited increased PAL activity at 36 hours, although at a lower level than the pathogen-treated seedlings. These findings are consistent with previous studies that have reported increased PAL activity in plants treated with beneficial bacteria and challenged with pathogens. Vanitha and Umesha (2011), reported maximum PAL activity in P. *fluorescens* DABBV4 pre-treated seedlings challenged with *R. solanacearum* at 12 hours after inoculation. Similarly, another study by Ramamoorthy *et al.* (2002) observed increased PAL activity in tomato roots treated with *P. fluorescens* Pf1 and challenged with *Fusarium oxysporum* f. sp. *lycopersici*. PAL induction has been observed in various plants, including patchouli and rice (Xie *et al.,* 2017; Nithya *et al.,* 2019). The enzyme PAL plays a crucial role in the production of phenolics and phytoalexins, contributing to plant defense responses. Studies have also shown that rhizobacteria colonization and salicylic acid production can activate PAL activity in plants. Additionally, interactions between rhizobacteria and bean roots have been shown to increase mRNA expression of PAL and chalcone synthase (Zdor and Anderson 1992), while the colonization of *P. aeruginosa* 7NSK2 in bean leaves led to increased PAL activity through enhanced salicylic acid production (De Meyer *et al.,* 1999).

C. Estimation of PPO

The PPO activity in tomato seedlings induced with *P. aeruginosa* OD13 and challenged with the pathogen after 36 hours of incubation was higher (44.33±1.25 Δ A420 nm/min/mg protein) compared to the control. However, the PPO activity in tomato seedlings treated solely with *R. solanacearum* (36.47±1.42 Δ A420 nm/min/mg protein) was slightly higher than in seedlings treated with *P. aeruginosa* OD13 alone (31.67±1.35 Δ A420 nm/min/mg protein) when compared to the control.

The current study observed an increase in PPO (polyphenol oxidase) activity in tomato seedlings induced with *P. aeruginosa* OD13 and challenged with *R. solanacearum* at 36 hours compared to control and other treatments. This increase in PPO activity contributes to the suppression of wilt disease in tomato plants, as PPO catalyzes the oxidation of phenolic compounds to more toxic quinones, which play a significant role in plant disease resistance. Previous studies support these findings, such as the work by Konappa *et al*. (2016), which reported a significant increase in PPO activity at 32 hours post-inoculation in lactic acid bacteria (LAB)-treated seedlings challenged with *R. solanacearum*. Xie *et al*. (2017) also observed high levels of PPO activity in upper leaves and lower stems at 48 hours post-inoculation in patchouli plants later challenged with *R. solanacearum*. Similarly, different treatments of rice seedlings inoculated with *R. solani* and treated with *Pseudomonas sp.* VSMKU2 showed increased PPO activity on the 7th day (Nithya *et al.*, 2019). Other studies, including those by Anand *et al*. (2007); Kavino *et al.* (2008); Ganeshamoorthi *et al.* (2008); Vanitha and Umesha (2011); Ashajyothi *et al*. (2020) demonstrated higher PPO activity in *Pseudomonas*-treated rice, banana and tomato plants challenged with *R. solanacearum*. The role of PPO in oxidizing phenolics to highly toxic quinones, thus halting disease development, has been reported in various plant species, including banana and tomato (Barilli *et al*., 2010; Thakker *et al.*, 2013; El-Argawy and Adss 2016). It has been suggested that the increase in PPO activity in elicitor-treated plants occurs gradually over time, indicating that plants have triggers to enhance PPO production (Thakker *et al.*, 2011; Thakker *et al.*, 2013).

D. Estimation of LOX

The tomato seedlings treated with *P. aeruginosa* OD13 and subsequently challenged with the pathogen displayed the highest level of LOX activity at 48 hours (49±1.42 Δ A234 nm/min/mg protein), which was significantly higher compared to the control. When only *P. aeruginosa* OD13 treatment was applied to the tomato seedlings, a moderate level of LOX activity (42.67±0.94 Δ A234 nm/min/mg protein) was observed. On the other hand, tomato seedlings treated with *R. solanacearum* alone showed a lower level of LOX activity (31±1.25 Δ A234 nm/min/mg protein) compared to the control.

LOX, an essential enzyme involved in plant growth and defense mechanisms against diseases, plays a crucial role in plant protection (Croft *et al.,* 1993; Vanitha and Umesha 2008). It initiates the plant's defense response by catalyzing the synthesis of various antimicrobial compounds. The products of LOX activity, known as phyto-oxylipins, are converted into jasmonic acid and other antimicrobial oxylipins, which are particularly prominent in Solanaceae plants, including tomatoes (Blee, 2002; Ongena *et al*., 2004). In our study, we observed maximum LOX activity in tomato seedlings that were pre-treated with *P. aeruginosa* OD13 and

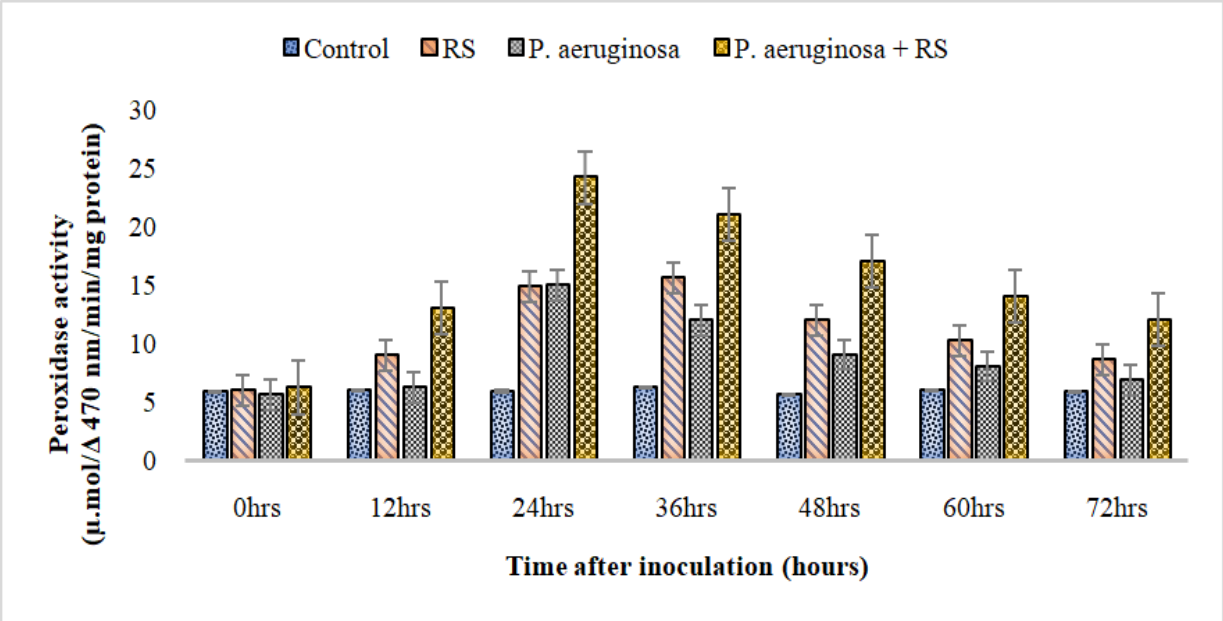
subsequently inoculated at 48 hours. Similar findings were reported by Vanitha and Umesha (2011), who found higher LOX activity at 9 hours post-inoculation in seedlings treated with *P. fluorescens* DABBV4 and challenged with *R. solanacearum*. Increased LOX activity in tomato plants treated with *P. putida* BTP1 and inoculated with *Bacillus cinerea* has been attributed to the upregulation of two LOX isoforms: TomLoxD and TomLoxF (Mariutto *et al*., 2011; Kurabachew and Wydra 2014). Previous studies have also demonstrated increased LOX activity in tomato leaf extracts following seed treatment with rhizobacteria (Silva *et al.,* 2004).

E. Total phenol content

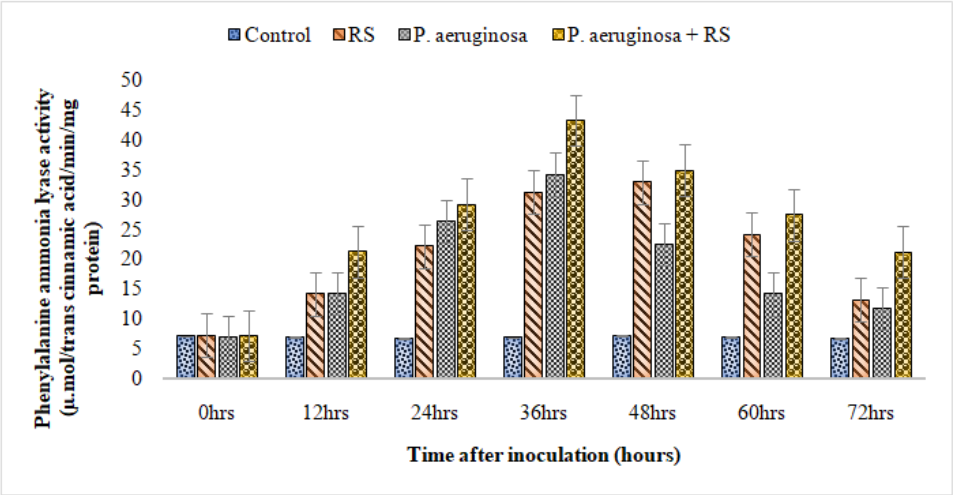
At 24 hours, the highest level of phenolic content (83.5±1.32 µg of catechol/min/mg protein) was observed in tomato seedlings treated with *P. aeruginosa* OD13 and subsequently challenged with *R. solanacearum*. Similarly, tomato seedlings treated with *P. aeruginosa* OD13 alone also showed significant phenolic content accumulation (70.5±0.98 µg of catechol/min/mg protein) compared to tomato seedlings inoculated with *R. solanacearum* alone (47.33±1.25 µg of catechol/min/mg protein).

Total phenol content was increased in the treatment of *P. aeruginosa* OD13 with challenge inoculation of tomato wilt pathogen *R. solanacearum* at 24h pre-treatments compared to control. At the same time moderate level phenol content was increased *P. aeruginosa* OD13 alone treated tomato seedling, however very less amount of phenol content was observed pathogen treated tomato seedlings compared to control. According to previous report our findings are coherence with the reports of (Anita and Samiyappan 2012), due to the induction of rhizobacteria rice roots secretes high amount of phenol when treated with Meloidogyne graminicola. Anand *et al*. (2007) stated that increase in total phenols content was remarkably very high in Pseudomonas-treated plants challenge inoculated with *A. solani* and *S. lycopersici* followed by azoxystrobin. Hence, phenolic contents are known to be important role for plant defence mechanism against various pathogens. Further, most of rhizobacterium and fungi secrete hydrolytic enzymes to develop phenolic contents and ooze out indole acetic acids, this process are mainly involved in phenol metabolism in plants.

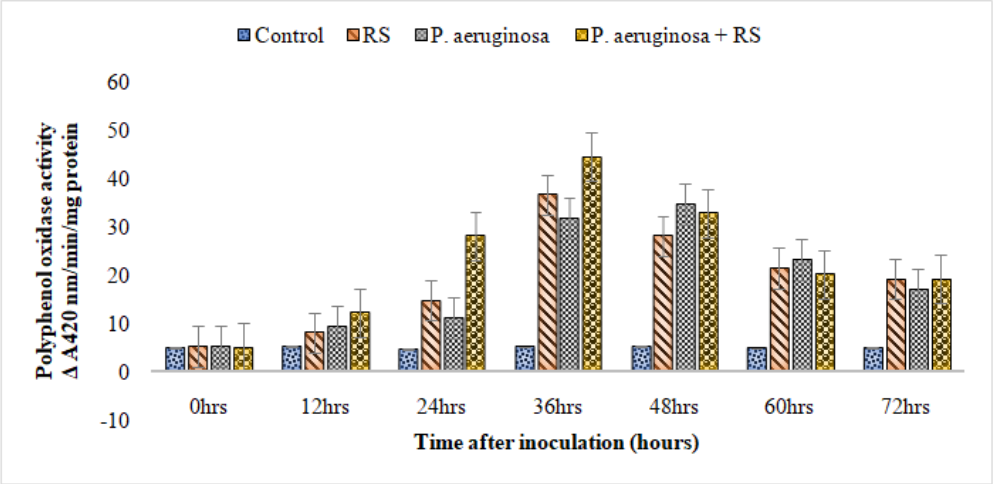
The total phenol content was significantly increased in tomato seedlings treated with *P. aeruginosa* OD13 and challenged with *R. solanacearum* at 24 hours compared to the control. Moderate levels of phenol content were also observed in tomato seedlings treated with *P. aeruginosa* OD13 alone, while pathogen-treated seedlings showed a lower amount of phenol content compared to the control. These findings are consistent with previous reports that indicate the induction of phenol secretion in rice roots treated with rhizobacteria when exposed to *Meloidogyne graminicola* (Anita and Samiyappan 2012). Also an increase in total phenol content had been observed in *Pseudomonas*-treated plants inoculated with *A. solani* and *S. lycopersici*, followed by azoxystrobin treatment (Anand *et al.,* 2007). Phenolic compounds play a crucial role in plant defense mechanisms against various pathogens. Rhizobacteria and fungi secrete hydrolytic enzymes and indole acetic acids, which are involved in phenol metabolism in plants.



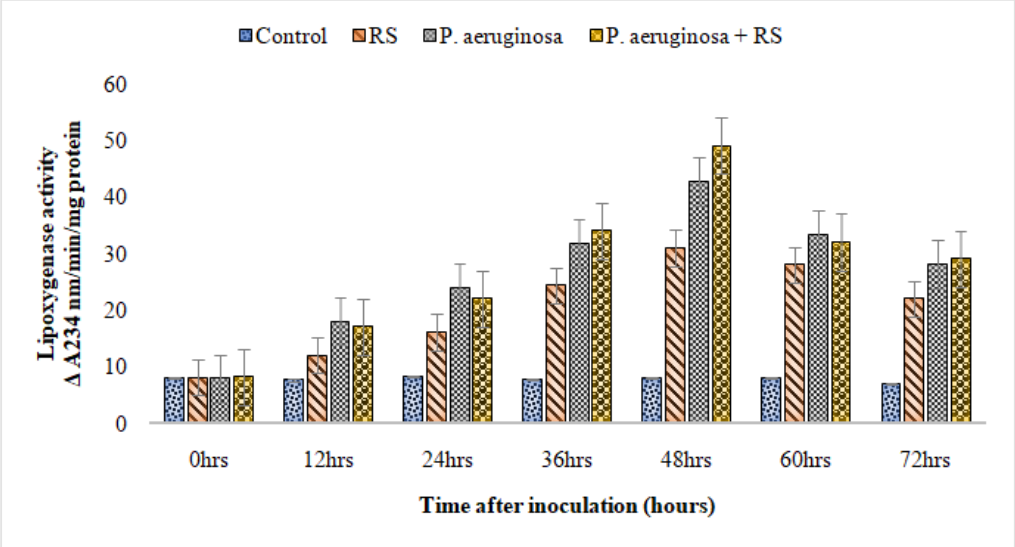
(A)


Control
RS
P. aeruginosa
P. aeruginosa + RS
Phenylalanine ammonia lyase activity (µ.mol/trans cinnamic acid/min/mg protein)
50
45
40
35
30
25
20
15
10
5
0
0hrs
12hrs
24hrs
36hrs
48hrs
60hrs
72hrs
Time after inoculation (hours)

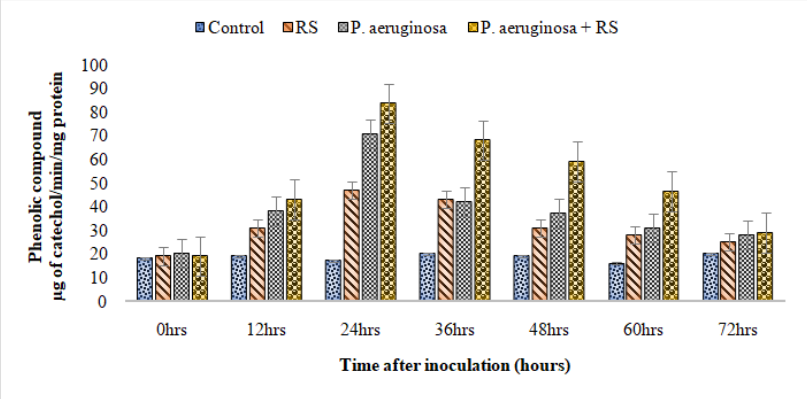
(B)


Control
RS
P. aeruginosa
P. aeruginosa + RS
Polyphenol oxidase activity Δ A420 nm/min/mg protein
60
50
40
30
20
10
0
-10
0hrs
12hrs
24hrs
36hrs
48hrs
60hrs
72hrs
Time after inoculation (hours)

(C)


Control
RS
P. aeruginosa
P. aeruginosa + RS
Lipoxygenase activity Δ A234 nm/min/mg protein
60
50
40
30
20
10
0
0hrs
12hrs
24hrs
36hrs
48hrs
60hrs
72hrs
Time after inoculation (hours)

(D)



(E)

Control (Tomato seedlings treated with sterile distilled water); *R. solanacearum* (RS) - (Tomato seedlings challenged with *R. solanacearum*); Culture (Tomato seedlings inoculated with *P. aeruginosa* OD13); Culture + RS (Tomato seedlings inoculated with *P. aeruginosa* OD13 + *R. solanacearum* at different time interval.

Fig. A. Peroxidase (POX) activity, **B**. Phenylalanine ammonialyase (PAL) activity, **C.** Polyphenol oxidase (PPO) activity, **D.** Lipoxygenase (LOX) activity, **E.** Total phenol content

CONCLUSIONS

Our findings suggest that *Pseudomonas aeruginosa* OD13 has the potential to induce ISR (Induced Systemic Resistance) and can be utilized as a bioinoculant for the management of bacterial wilt, caused by *Ralstonia solanacearum*, in tomato plants. The beneficial rhizobacterium exhibits a protective mechanism against soil-borne pathogens, making it a promising candidate for disease control in tomato cultivation. By harnessing the ISR pathway, *P. aeruginosa* OD13 enhances the plant's natural defense system, providing an effective means to combat bacterial wilt. These results highlight the potential of *P. aeruginosa* OD13 as a biocontrol agent, offering sustainable and environmentally friendly solutions for tomato crop protection.

Acknowledgements. We are thankful to Department of Plant Pathology, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha for providing the facility to conduct the study.

Conflict of Interest. None.

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How to cite this article: Anjali Suansia and Akshaya Kumar Senapati (2022). Association of some Plant Defense Enzyme activities with Systemic Resistance to Bacterial wilt Disease induced in Tomato Plants by *Pseudomonas aeruginosa* Strain OD13. *Biological Forum – An International Journal*, *14*(4): 1342-1349.