Contributions of Central Research Institute for Agriculture
Vol. 18, No. 4, October 2024, pp. 154~161
ISSN: 2962-9217, DOI: 10.59651/ccria
Published by: Central Research Institute for Agriculture (CeRIA)

Salicylic Acid Accumulation in Tomato Root Induced by Endopytic Bacteria and Exogenous Salicylic Acid Response to *Ralstonia syzygii* subsp. *indonesiensis* Infection

Triwanto Nababan[1*], Lisnawita[2], Irda Safni[2]
[1] Magister Program of Agrotechnology, Faculty of Agriculture, Universitas Sumatera Utara, Medan, Indonesia.
[2] Program Study of Agrotechnology, Faculty of Agriculture, Universitas Sumatera Utara, Medan, Indonesia.

Article Info

Article history:

Received : May 09, 2024
Revised : Jul 13, 2024
Accepted : Sept 20, 2024

Keywords:

Arthrobacter sp;
Bacillus thuringiensis;
Bacterial wilt;
Induce resistance.

ABSTRACT

Ralstonia syzygii subsp. *indonesiensis* causes bacterial wilt disease is a soil-borne pathogen that causes serious damage and major losses in tomato production. To overcome this, the use of *Arthrobacter sp.* and *Bacillus thuringiensis* as biological elicitors and salicylic acid as a chemical elicitor were teste their capacity to induce tomato plants to become resistant. Experiments were carried out on the susceptible tomato cultivar "Servo F1" in sterilized soil to test the elicitor's effectiveness in triggering plant defense mechanisms in response to salicylic acid accumulation in afflicted roots. *Arthrobacter* sp. and salicylic acid treatments significantly reduced the disease severity due to bacterial wilt compared to control treatment within three weeks after inoculation. Tomato with once week application intensity was also better than every two weeks application intensity. The AUDPC value showed by *Arthrobacter* sp. was 1449.7 with an application intensity every two weeks and 148 with an application intensity once a week compared to control with an AUDPC value of 4962.9. Furthermore, endophytic bacteria and salicylic acid can induce salicylic acid accumulation in pathogen-inoculated tomato roots. The results show that the elicitor is either biological or chemicals play an important role as inducers of plant defenses, thereby reducing bacterial wilt disease.

Corresponding Author:

Triwanto Nababan
Magister Program of Agrotechnology, Faculty of Agriculture, Universitas Sumatera Utara
Jl. Dr. T. Mansur No.9, Padang Bulan, Kec. Medan Baru, Kota Medan, Sumatera Utara 20222
Email : triwannababan31@gmail.com

1. INTRODUCTION

In field conditions, plants will always be attacked either by environmental conditions or pests and diseases (Carrer et al., 2016). Moreover, tomato plants (*Solanum lycopersicum*) are susceptible to disease infections such as *Ralstonia syzygii subsp. indonesiensis* which causes bacterial wilt disease. These pathogens are soil-borne and cause serious damage to economic yields (Wenas et al., 2017; Rahmadhani et al., 2022). Hundreds of different plant species can be attacked by this genus of pathogen (Genin, 2010). Every year, there is a growing need for tomatoes in Indonesia and across the globe, but the challenge of wilt disease is the main obstacle. Therefore, sustainable and environmentally friendly tomato production needs to be carried out to support economic growth and the world's need for tomatoes (Chaudrary et al., 2019). Plants are naturally able to identify invasive pathogenic microbes and initiate defensive mechanisms in response (Pandey et al., 2017). According to Klopper et al. (1992) There are two types of plant systemic resistance: acquired systemic resistance (SAR) and induced systemic resistance (ISR). SAR pathway required salicylic acid (SA) as initiating signal molecule (Buensanteai et al., 2010) and ISR pathway depend on jasmonic acid (JA) and ethylene (ET) as signaling factors mediated by useful microorganisms as elicitors (Walters et al., 2007). Endophytic bacteria and SA have been used in research as elicitors to increase plant resistance and control various plant pathogens, like *Ralstonia solanacearum* and *Meloidogyne* spp. (Elsharkawy et al., 2015), *Fusarium solani* (Saengchan et al., 2022), *Fusarium oxysporum (*Lastochkina et al., 2020) and *Pseudomonas syringae* (Zhang et al., 2021).

Exogenous SA can increase the production of plant defense enzyme activity related to pathogenesis proteins (PR) in the defense response of solanaceae impaired due to physiological and biochemical imbalances (Mostafanezhad et al., 2014) and also directly act as a growth regulator in plants. Endophytic bacteria will colonize the roots and trigger the activation of biochemical and physiological defense systems to encourage increased growth (Buensanteai et al., 2012). Together with our understanding that endophytic bacterial inoculation and pathogen infection result in the endogenous SA accumulation in plants (Lastochkina et al., 2020). SA accumulation increases in areas of infection and will spread systemically to all parts of the plant as a defense response (Song et al., 2004). This research could be a model of the relationship between SA accumulation induced by endophytic bacteria or SA and pathogen infection signals. It remains unclear whether the role of SA accumulation in increasing plant resistance is mediated by the two types of chemical and biological elicitors or which systemic resistance pathways result in endogenous SA accumulation under pathogen infection conditions.

In this study, we used *Arthrobacter* sp. and *Bacillus thuringiensis* as biological elicitors and exogenous SA as a chemical elicitor to induce plant resistance and investigate SA accumulation in tomato roots under *R. syzygii* subsp. *indonesiensis* infection. We hypothesize that endophytic bacteria and SA can increase plant resistance by increasing the accumulation of SA as a defense response in afflicted roots.

2. METHOD

2.1 Plant Material

Tomato plant cv "Servo F1" was grown in a tray filled with sterilized humus soil. After 1 weeks, the plants were moved using a soil and compost mixture (3:1) into pots (15cm) which were sterilized with steamer 90°C-121°C for 8 hours (Sujoko et al., 2015) and grown at 28°C under greenhouse condition.

2.2 Growth Condition of *R. syzygii* subsp. *indonesiensis* and Endopytic Bacteria

Patogen *R. syzygii* subsp. *indonesiensis* was isolated from tomato plants with wilting symptoms in Dolat Rayat District, Karo Regency, North Sumatera. *R. syzygii* subsp. *indonesiensis* was cultured at 30°C on Tryphenyl tetrazolium chloride (TZC) semi selective medium containing five milliliter 1% TZC, 10g peptone, 1g casein hydrolysate, 5g glucose, 12g agar in 1L distilled water for 48 h. (Safni *et al.,* 2014). The pathogens cells were suspended in sterile distilled and centrifugation at 7000 rpm at 25°C for 10 minute and adjust to a fial density of 10^8cfumL^{-1}.

The endophytic bacteria used are *Arthrobacter* sp. and *B. thuringiensis* the Plant Disease Laboratory, Faculty of Agriculture, Universitas Syiah Kuala, Aceh. Nutrient Agar (NA) medium was used to cultivate the isolates, and subsequently incubated for 48 hours at 28°C. The distilled water was sterile and used to suspend the bacterium cells and centrifugation at 7000 rpm at 25°C for 10 minute and adjust to a final density of 10^8cfumL^{-1}.

2.3 Exogenous SA Preparation

SA obtained from the Plant Disease Laboratory, Universitas Sumatera Utara, Indonesia. SA was dissolved in 95% ethanol and sterile distilled water and adjusted to 10 mM (Leiwakabessy et al., 2017).

2.4 Experimental Design

Completely randomized design has been used in the study used eight treatment levels, namely:

K0– = Control without *R. syzygii* subsp. *indonesiensis*
K0+ = Control with *R. syzygii* subsp. *indonesiensis*
AB1 = *Arthrobacter* sp. once a week
AB2 = *Arthrobacter* sp. once every two weeks
BT1 = *B. thuringiensis* once a week
BT2 = *B. thuringiensis* once every two weeks
SA1 = Salicylic acid once a week
SA2 = Salicylic acid once every two weeks

Each treatment was tested on tomato plants planted in 15 cm pots and repeated three times, For each replication, three plants were prepared and 73 experimental sample units for total. Endophytic bacteria and salicylic acid treatment was given to tomato plants aged one week after transplanting. Tomato rizhosfer were irrigated with 100ml culture medium. One week after treatment plants were inoculated using root cuttings and soil drench of 100 mL (10^8 cfu.mL^{-1}) of *R. syzygii* subsp. *indonesiensis* suspension into each pot.

2.5 Evaluate AUDPC Bacterial Wilt Disease

Following the pathogen inoculation, the Bacterial wilt Disease AUDPC was observed for 14 days based on wilting symtoms. Assessed according to Winstead and Kelman (1952) using 0–5 scale: 0, healthy plants; 1, wilting of one lower leaf in portion; 2, wilting of 2–3 lower leaf; 3, all save the top two or three leaf wilt; 4, withering of every leaf; and 5, dead plants. The AUDPC value was calculated to see the development of wilt disease symptoms in each treatment The AUDPC formula (1) is calculated using the Van der Plank formula (1963):

$$\mathrm{AUDPC} = \sum_{i}^{n-1} \left(\frac{\mathrm{yi} + \mathrm{yi} + 1}{2}\right)(\mathrm{ti} + 1 - \mathrm{ti}) \qquad (1)$$

Description: yi+1 = Observation data to-i +1; yi = Observation data to-i ; ti +1 = Observation time to-i +1; ti = Observation time to-i.

2.6 Extraction and Determination of SA Accumulation

Extraction is carried out with Nair and Umamaheswaran (2016) method. SA was extracted from 0.5 g of tomato roots and Pulverized in a cold mortar using liquid nitrogen and 1% of polyvinyl pyrrolidone (PVP) as an internal standard, and the mixture was homogenized in 3 mL sodium phosphate buffer (0.1 M, pH 6.5). Suspense was centrifuged at 5000 rpm at 4°C for 25°C. The supernatant used for the assay of SA accumulation. The stock solution used is $FeCl_3$ made with a 0.1% standard solution with a concentration of 0.1 mg.mL^{-1}. Next, the supernatant was mixed with $FeCl_3$ solution with an absorbance at 540 nm (Warrier et al., 2013).

2.7 Statistics Analysis

Data were evaluated by analysis of variance (ANOVA) means were compared using Duncan's multiple comparison 5% (SPSS 25).

3. RESULTS AND DISCUSSION

3.1 Effect of Endopytic Bacteria and SA on The Severity of Bacterial Wilt Disease

Plant disease intensity over time is quantified by the AUDPC value. In this study, the AUDPC value was influenced by endophytic bacteria and also by SA which showed a more significant effect on development caused by *R. syzygii* subsp. *indonesiensis*. Tomato positive control (Fig. 1a) with an AUDPC value of 4962.9. Application intensity is an essential component in the development of resistance where it showed by *Arthrobacter* sp. in Figure 1b the AUDPC value is 1449.7 with an application intensity once every two weeks and 148 with an application intensity once a week. Likewise with the other two single treatments B*. thuringiensis* (Fig. 1c) and SA (Fig. 1d).

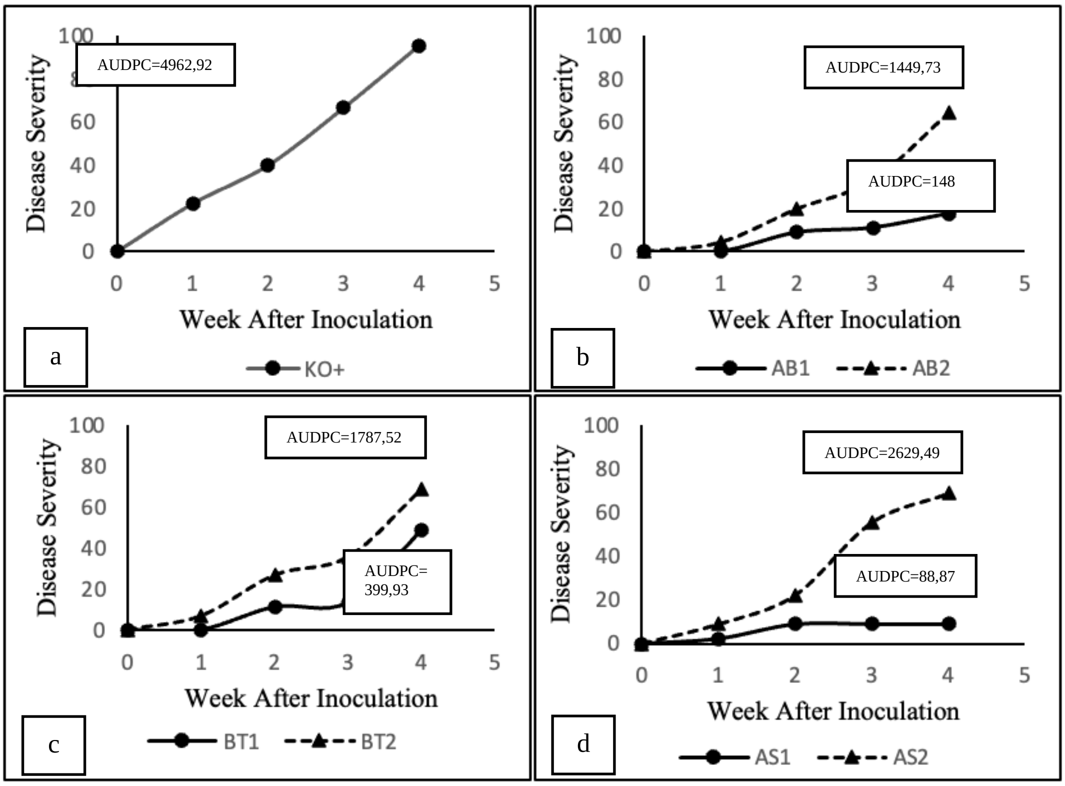

Figure 1. Graphical interpretation of AUDPC calculations of wilt disease caused by the pathogen *R. syzygii* subsp. *indonesiensis.* (a) *R. syzygii* subsp. *indonesiensis*; (b) *Arthrobacter* sp*;* (c) *B. thuringiensis;* (d) Exogenous SA;

Tomato treated with *Arthrobacter* sp. and SA once a week has a good ability to inhibit bacterial wilt disease, characterized by a low Graphical interpretation of AUDPC calculations of wilt disease during two observations. These results are in line with Velázquez et al. (2013) *Arthrobacter agilis* is able to suppress *botrytis cinerea* and also induce exogenous SA in cassava plants infected with *Fusarium solani* (Saengchan et al., 2021). Aspirin, 2,6-dichloroisonicotinic acid (INA), and benzothiadiazole S-methyl ester (BTH) are some instances of exogenous SA applications which enhance PR gene expression and resistance, which can complement their increased disease resistance phenotype (Cordova et al., 2012). These results have been found in Arabidopsis disease resistance and defense response activation (Caruana et al., 2020), *P. syringae* in tomato (Uppalapati et al., 2007) and biotic and abiotic stress in tobacco (Catinot et al., 2008). The majority agree that SA signaling and plant resistance to hemibiotrophic and biotrophic diseases are associated (zhang et al., 2018). Endopytic bacteria stated the capacity to boost plant resilience by increasing the levels of SA and JA in plants and activating the SA and JA/ET signaling pathways, respectively (Hyakumachi et al., 2013; Pieterse et al., 2014; Aviles et al., 2016).

The secondary compounds that endophytic bacteria produce are able to directly oppose pathogens and function as immune elicitors to trigger the ISR pathway. (Prsic and Ongena, 2020). Secondary metabolite compounds produced such as Phenazines (Chin et al., 2003), polysaccharides (EPS) (Jiang et al., 2016), Harzianic acid (Manganiello et al., 2018), Microbial volatile compounds (MVCs) (Kong et al., 2018) and Cyclic lipopeptides surfactin and VOC 2, 3-butanediol (Ryu et al., 2004; Chowdhury et al., 2015) as molecules, triggered the activation phytohormones SA and JA of signal transduction pathways. These reveal how endophytic bacteria and plants interact: the bacteria secrete substances known as secondary metabolites to increase the resistance of the host plants, while the plants create metabolites to attract the bacteria to avoid pathogen infection.

Bacterial wilt disease progression curve is a monomolecular model curve that will be used to calculate the AUDPC value. This finding supported by Madden et al. (2007) who reported that the monomolecular model was in better statistical agreement with other statistical models of plant diseases including estimation of epidemiological rates, disease severity and determination of disease incidence. Treatment with *Arthrobacter* sp. and SA had the lowest AUDPC value and the lowest disease development rate, while *B. thuringiensis* had the highest AUDPC value and disease development rate. Tomato plants are very susceptible to wilt infection, as explained by Lebeau et al., (2011) that while potato, tomato, and eggplant are all members of the same family, their responses to with *R. syzygii* species *complex* (RSSC) strains varied, indicating that potato and eggplant have a high level of defense against bacterial wilt, in contrast to tomatoes.

3.2 SA Accumulation from Tomato Roots

The results of SA accumulation are presented in Figure 2. Higher SA accumulation was found in SA1 treatment with a value of 240.86 ng/g, which was not significantly different from the SA accumulation in the *Arthrobacter* sp. (AB1) and *B. thuringiensis* (BT1) once a week applied intensity, with SA accumulation 203.75 ng/g and 214.26 ng/g.

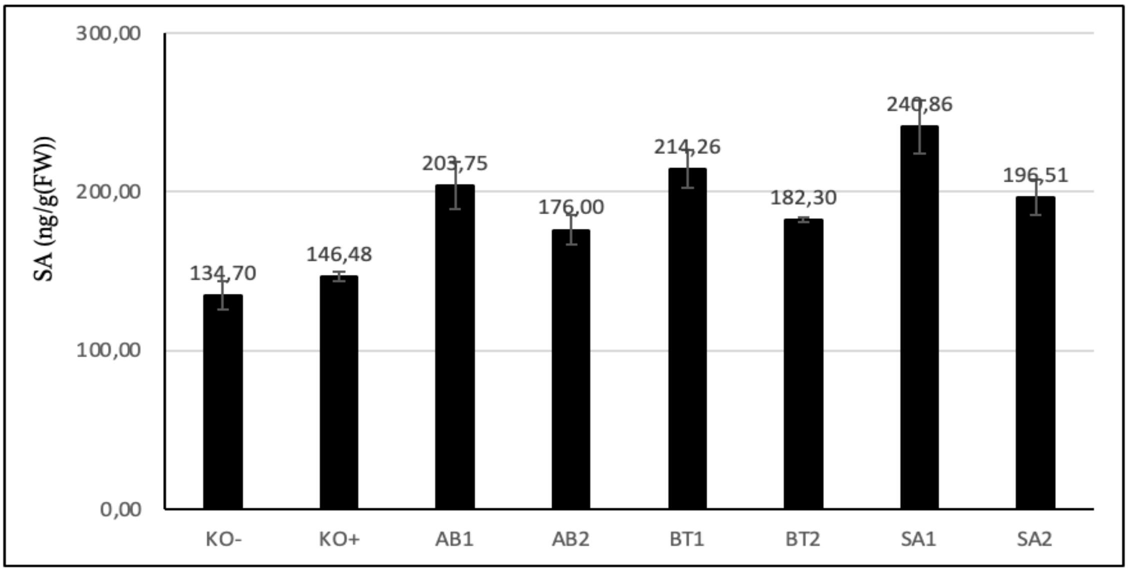

Figure 2. SA accumulation in tomato roots after treatment with endophytic bacteria, SA and the pathogen *R. syzygii* subsp. *indonesiensis* infection. *Data calculated in ng x10^3. K0–= without *R. syzygii* subsp. *indonesiensis;* K0+= with *R. syzygii* subsp. *indonesiensis;* AB1= *Arthrobacter* sp. once a week; AB2 = *Arthrobacter* sp. once every two weeks; BT1= *B. thuringiensis* once a week; BT2= *B. thuringiensis* once every two weeks; SA1= SA once a week; SA2= SA once every two weeks.

In this study, the results showed that the application of endophytic bacteria and SA could induce tomato resistance to the soil-borne pathogen *R. syzygii* subsp. *indonesiensis.* The high content level of SA accumulation in tomato roots treated with *Arthrobacter* sp. and *B. thuringiensis* are related to the level of tomato resistance to infection with *R. syzygii* subsp *indonesiensis*. According to Silverman et al. (1995) The expression of SA, which has a positive correlation with the SA accumulation content, determines the level of plant resistance. SA functions in the elicitation and signaling aspects of the systemic resistance-inducing pathway. In response to oxidative stress, necrosis, and mechanical injury, SA is produced at the elicitation level and subsequently distributed throughout the plant (Heil and Bostock, 2002).

Application of exogenous SA to the rhizosphere resulted in a considerable improvement in the level of SA accumulation. It's potential that the root system of tomatoes can absorb SA, disseminate it throughout the plant, and eventually boost resistance against systemic diseases (Spletzer et al., 1999). Tobacco plants treated with SA also exhibited a comparable reaction (Oostendorp *et al.,* 2001) and SA accumulation in rice treated with exogenous SA under *Xanthomonas oryzae* infection (Le Thanh et al., 2017). One factor lowering tomato wilt disease is an increase SA accumulation in afflicted areas. In addition, the SA accumulation is associated with the defense mechanism that tomato utilized, which is triggered by an inducer agent and SA as an elicitor.

4. CONCLUSION

This study showed a decrease in the severity of bacterial wilt disease due to once-weekly application of endophytic bacteria and SA are led to increased SA accumulation in the tomato roots under *R. syzygii* subsp. *indonesiensis* infection which was a sign of activation of systemic resistance. Further research needs to be developed considering that many endophytic bacteria have the capacity to promote plant resistance and can reduce the use of pesticides to support sustainable environmentally friendly agriculture.

REFERENCES

Aviles, G. M. E., Flores-Cortez, I., Hernández-Soberano, C., Santoyo, G., and Valencia- Cantero, E. (2016). The plant growth promoting rhizobacterium *Arthrobacter agilis* UMCV2 endophytically colonizes Medicago truncatula. Rev. Argent. Microbiol. 48 : 342–346.

Buensanteai, N., Mukherjee, P. K., Horwitz, B.A., Cheng, C., Dangott, L. J., Kenerley, C.M. (2010). Expression and purification of biologically active *Trichoderma virens* proteinaceous elicitor Sm1 in pichia pastoris. Protein Express Purif. 72:131–138.

Buensanteai, N., Thumanu, K., Sompong, M., Athinuwat, D., and Prathuangwong, S. (2012). The FTIR spectroscopy investigation of the cellular components of cassava after sensitization with plant growth promoting rhizobacteria, *Bacillus subtilis* CaSUT007. Afr J Microbiol Res. 6: 603–610.

Carrer F, R., Oliveira, R.M., Dias, V.D., Rocha, G.A., Dianese, E´.d.C., and Cunha, M.G.d. (2016). Selection of tomato accessions resistant to Verticilium wilt. Pesqui. Agropecu´aria Trop. 46 : 429–433.

Caruana, J.C., Dhar, N. and Raina, R. (2020). Overexpression of *Arabidopsis* microRNA167 induces salicylic acid-dependent defense against *Pseudomonas syringae* through the regulation of its targets ARF6 and ARF8. *Plant Direct. 4* : 02- 70.

Catinot, J., Buchala, A., Abou-Mansour, E. and Me´ traux, J.P. (2008). Salicylic acid production in response to biotic and abiotic stress depends on isochorismate in *Nicotiana benthamiana*. *FEBS Lett.* 5(82) : 473–478.

Chaudhary, J., Alisha, A., Bhatt, V., Chandanshive, S., Kumar, N., Mir, Z., Kumar, A., Yadav, S.K., Shivaraj, S.M., Sonah, H., and Deshmukh, R. (2019). Mutation breeding in tomato: Advances, applicability, and challenges. Plants 8 (5): 128.

Chin-A-Woeng, T.F.C., Bloemberg, G.V., and Lugtenberg, B.J.J. (2003). Phenazines and their role in biocontrol by *Pseudomonas* bacteria. *New Phytol. 157*: 503–523.

Chowdhury, S.P., Uhl, J., Grosch, R., Alquéres, S., Pittroff, S., Dietel, K., Schmitt-Kopplin, P., Borriss, R. and Hartmann, A. (2015). Cyclic Lipopeptides of *Bacillus amyloliquefaciens* subsp *plantarum* Colonizing the Lettuce Rhizosphere Enhance Plant Defense Responses Toward the Bottom Rot Pathogen *Rhizoctonia solani. Mol. Plant Microbe Interact. 28* : 984–995.

Cordova, C, O., Adame-Alvarez, R. M., Acosta-Gallegos, J. A., and Heil, M. (2012). Domestication affected the basal and induced disease resistance in common bean (*Phaseolus vulgaris*). *Eur. J. Plant Pathol.* 134: 367–379.

Elsharkawy, M. M., Nakatani, M., Nishimura, M., Arakawa, T., Shimizu, M., and Hyakumachi, M. (2015). Control of tomato bacterial wilt and root-knot diseases by *Bacillus thuringiensis* CR-371 and *Streptomyces avermectinius* NBRC14893. *Acta Agriculturae Scandinavica, Section B—Soil & Plant Science*. *65*(6): 575-580.

Genin, S. (2010). Molecular traits controlling host range and adaptation to plants in *Ralstonia solanacearum*. *New Phytol. 187* : 920–928.

Heil, M and Bostock, R. M. (2002). Induced systemic resistance (ISR) against pathogens in the context of induced plant defences. *Ann. Bot.* 89(5): 503– 512.

Hyakumachi, M., Nishimura, M., Arakawa, T., Asano, S., Yoshida, S., Tsushima, S., and Takahashi, H. (2013). *Bacillus thuringiensis* suppress bacterial wilt disease caused by *Ralstonia solanacearum* with systemic induction of defense-related gene expression in tomato. *Microbe Environ.* 28:128–134.

Jiang, C.H., Fan, Z.H., Xie, P., and Guo, J.H. (2016). *Bacillus cereus* AR156 Extracellular Polysaccharides Served as a Novel Micro-associated Molecular Pattern to Induced Systemic Immunity to *Pst* DC3000 in *Arabidopsis*. *Front. Microbiol. 7* : 664.

Kloepper, J.W., Tuzun, S., and Kuc, J.A. (1992). Proposed definitions related to induced disease resistance. *Biocontrol Sci. Technol. 2* : 349–351.

Kong, H. G., Shin, T. S., Kim,T. H. and Ryu, C. M. (2018) Stereo isomers of the Bacterial Volatile Compound 2,3-Butanediol Differently Elicit Systemic Defense Responses of Pepper against Multiple Viruses in the Field. *Front. Plant Sci. 9* : 90.

Lastochkina, O., Pusenkova, L., Garshina, D., Yuldashev, R., Shpirnaya, I., Kasnak, C., and Aliniaeifard, S. (2020). The effect of endophytic bacteria *Bacillus subtilis* and salicylic acid on some resistance and quality traits of stored Solanum tuberosum L. tubers infected with fusarium dry rot. *Plants*. *9*(6), 738.

Le Thanh, T., Thumanu, K., Wongkaew, S., Boonkerd, N., Teaumroong, N., Phansak, P, and Buensanteai, N. (2017). Salicylic acid-induced accumulation of biochemical components associated with resistance against *Xanthomonas oryzae* pv. *oryzae* in rice. *J Plant Interact*. 12(1):108–120.

Lebeau, A., Daunay, M. C., Frary, A., Palloix, A., Wang, J. F., Dintinger, J., Chiroleu, F., Wicker, E., and Prior, P. (2011). Bacterial wilt resistance in tomato, pepper, and eggplant: Genetic resources respond to diverse strains in the *Ralstonia solanacearum* species *complex. Phytopathology* 101:154-165

Leiwakabessy, C., Sinaga, M. S., Mutaqin, K. H., Trikoesoemaningtyas, T., and Giyanto, G. (2017). Asam salisilat sebagai penginduksi ketahanan tanaman padi terhadap penyakit hawar daun bakteri. *Jurnal Fitopatologi Indonesia*, *13*(6) : 207-207.

Madden, L. V., Hughes, G., and van den Bosch, F. (2007). *The study of plant disease epidemics*. St. Paul, USA: American Phytopathological Society (APS Press).

Manganiello, G., Sacco, A., Ercolano, M.R., Vinale, F., Lanzuise, S., Pascale, A., Napolitano, M., Lombardi, N., Lorito, M., and Woo, S.L. (2018). Modulation of Tomato Response to *Rhizoctonia solani* by *Trichoderma harzianum* and Its Secondary Metabolite Harzianic Acid. *Front. Microbiol. 9* : 19-66.

Mostafanezhad, H., Sahebani, N. and Zarghani, S. N. (2014). Control of root-knot nematode (*meloidogyne javanica*) with combination of *arthrobotrys oligospora* and salicylic acid and study of some plant defense responses. *Biocontrol Sci Technol.* 24(2):203–215.

Nair, A.B. and Umamaheswaran, K. (2016). Enzymatic Responses to SriLankan cassava mosaic virus infection in cassava plants after grafting. *Int J Appl Pure Sci Agric* 2:165–170

Oostendorp, M., Kunz, W., Dietrich, B., and Staub, T. (2001). Induced disease resistance in plants by chemicals. *J. Plant Pathol*. 107 : 19–28.

Pandey, P., Irulappan, V., Bagavathiannan, M.V. and Senthil-Kumar, M. (2017). Impact of Combined Abiotic and Biotic Stresses on Plant Growth and Avenues for Crop Improvement by Exploiting Physio-morphological Traits. *Front. Plant Sci. 8* : 537.

Pieterse, C. M., Zamioudis, C., Berendsen, R. L., Weller, D. M., Van Wees, S. C. and Bakker, P. A. (2014). Induced systemic resistance by beneficial microbes. *Ann Rev Phytopathol.* 52: 347–375.

Prsic, J. and Ongena, M. (2020). Elicitors of Plant Immunity Triggered by Beneficial Bacteria. *Front. PlantSci. 11*: 594-530.

Rahmadhani, N., Pinem, M. I., and Safni, I. (2022). Spread of bacterial wilt disease on potato in three villages in Silimakuta District, Simalungun, North Sumatra, Indonesia. *J Trop Plant Pests Dis*, *22*, 162-167.

Ryu, C, M., Farag, M.A., Hu, C.-H., Reddy, M.S., Kloepper, J.W. and Paré, P.W. (2004) Bacterial volatiles induce systemic resistance in *Arabidopsis*. *Plant Physiol. 134*: 1017–1026.

Saengchan, C., Sangpueak, R., Le Thanh, T., Phansak, P., and Buensanteai, N. (2022). Induced resistance against *Fusarium solani* root rot disease in cassava plant (*Manihot esculenta Crantz*) promoted by salicylic acid and *Bacillus subtilis*. *Acta Agriculturae Scandinavica, Section B—Soil & Plant Science*, *72*(1), 516-526.

Safni, I., Cleenwerk, I., De Vos, P., Fegan, M., Sly, L., and Kappler, U. (2014). Polyphasic taxonomic revision of the *Ralstonia solanacearum* spesies complex: Proposal to emend the description of *Ralstonia solanacearum* and Ralstonia syzygii and reclassify current *R. syzygii* strains as *Ralstonia syzygii* subsp. *syzygii* subsp. nov., *R. sonalacearum* phylotipe IV strains as *Ralstonia syzygii* subsp. *indonesiensis* subsp. nov., Banana Blood Disease Bacterium strains as *Ralstonia syzygii* subsp. *celebesensis* subsp. nov., and *R. solanacearum* phylotipe I and III strains as *Ralstonia pseudosolanacearum* sp. nov. *International J. of Systematic and Evolutionary Microbiology*. 64: 3087-3103.

Silverman, P., Seskar, M., Kanter, D., Schweizer, P., and Metraux, J. (1995). Salicylic acid in rice (biosynthesis, conjugation, and possible role). *Plant Physiol.* 108: 633-639.

Song, J.T., Lu, H., McDowell, J.M. and Greeberg, J.T. (2004). A key role for *ALD1* in activation of local and systemic defenses in *Arabidopsis*. *Plant J.* 40, 200–212.

Spletzer, M.E., and Enyedi, A.J. (1999). Salicylic acid induces resistance to *Alternaria solani* in hydroponically grown tomato, *Phytopathology* 89 : 722–727.

Sujoko, Ahmad., Mustofa, L., and Dwi, P. (2015). Kajian sterilisasi media tumbuh jamur tiram putih *(Pleurotus Ostreatus (L) Fries)* menggunakan steamer baglog." *Jurnal Keteknikan Pertanian Tropis dan Biosistem* 3(3) : 303-314.

Uppalapati, S.R., Ishiga, Y., Wangdi, T., Kunkel, B.N., Anand, A., Mysore, K.S. and Bender CL (2007) The phytotoxin coronatine contributes to pathogen fitness and is required for suppression of salicylic acid accumulation in tomato inoculated with *Pseudomonas syringae* pv. tomato DC3000. *Mol. Plant-Microbe Interact.* 20 : 955–965.

Van der Plank, J. E. (1963). *Plant diseases*. Elsevier Science.

Velázquez-Becerra, C., Macías-Rodríguez, L. I., López-Bucio, J., Flores-Cortez, I., Santoyo, G., Hernández-Soberano, C., and Valencia-Cantero, E. (2013). The rhizobacterium *Arthrobacter agilis* produces dimethylhexadecylamine, a compound that inhibits growth of phytopathogenic fungi in vitro. *Protoplasma*. *250*: 1251-1262.

Walters D, Newton A, Lyon G. (2007). Induced resistance for plant defence. UK: Blackwell Publishing.

Warrier, R. R., Paul, M., and Vineetha, M. V. (2013). Estimation of salicylic acid in Eucalyptus leaves using spectrophotometric methods. *Genetics and plant physiology*. *3*(1-2): 90-97.

Wenas, M., Manengkey, G. S. J, and Makal, H. V. G. (2017). Insidensi penyakit layu bakteri pada tanaman kentang (*Solanum tuberosum* L.) di Kecamatan Modoinding [The incidence of bacterial wilt disease in potato plants (*Solanum tuberosum* L.) in District of Modoinding]. *Cocos*. 7(3): 1–11.

Winstead, N. N. and Kelman, A (1952). Inoculation techniques for evaluating resistance to *Pseudomonas solanacearum. Phytopathology.* 42: 628-634.

Zhang, Z., Long, Y., Yin, X., and Yang, S. (2021). Sulfur-induced resistance against *Pseudomonas syringae* pv. actinidiae via triggering salicylic acid signaling pathway in kiwifruit. *International Journal of Molecular Sciences*, *22*(23), 12710.

Zhang, W., Zhao, F., Jiang, L., Chen, C., Wu, L., and Liu, Z. (2018). Different Pathogen Defense Strategies in *Arabidopsis* : More than Pathogen Recognition. *Cells. 7* : 252