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Secondary metabolites of the granular form of *Pseudomonas fluorescens* P60 and its applications to control tomato bacterial wilt

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Abstract. *Soesanto L, Saputra DA, Sastyawan MWR, Mugiastuti E, Suprapto A, Rahayuniati RF. 2023. Secondary metabolites of the granular form of* Pseudomonas fluorescens *P60 and its applications to control tomato bacterial wilt. Biodiversitas 24: 2475-2482.* Bacterial wilt is one of the important diseases of tomatoes caused by *Ralstonia solanacearum* (Smith 1896) Yabuuchi et al. 1996. Its control can be achieved by applying secondary metabolites of *Pseudomonas fluorescens* Migula 1895 P60 in granular form. The study aimed to determine the shelf life of the secondary metabolites *P. fluorescens* P60' in granular form and its application to manage bacterial wilt and the performance of tomatoes. The study was conducted for five months at the Plant Protection Laboratory and Experimental Farm, Faculty of Agriculture, Universitas Jenderal Soedirman. A randomized block design was used with four replicates and six treatments, consisting of R. solanacearum (control), R. solanacearum + 1, 5, 10, or 15 g, and bactericide (a.i. agrimycin sulphate 20%). Variables observed were inhibition zone, incubation period, disease intensity, the area under disease progress curve (AUDPC), crop height, crop fresh and dry weight, root fresh and dry weight, and phenolic compounds qualitatively. Results of the research showed that the granular formula until the 4th week still had an inhibition zone between 21.67-23.34%. However, the granules stored for as much as 15 g for four weeks effectively reduced disease intensity by 75.00%, decreased AUDPC value by 74.72%, and increased plant height, root dry weight, and fresh crop weight as well 44, 62.5, and 65.65 %, respectively. In addition, the granular formula increased the content of tomato phenolic compounds qualitatively.

Keywords: Bacterial wilt, granular formula, *Pseudomonas fluorescens* P60, secondary metabolites, tomato

INTRODUCTION

Tomato (*Lycopersicon esculentum* Miller) is one of the important horticultural commodities in Indonesia, even in the world (BPS 2020; Burton-Freeman and Reimers 2011). Regular consumption of tomatoes can prevent the formation of stones in the urinary tract, jaundice, constipation, and cancer (Agrawal and Singh 2009). Aside from being a vegetable, tomatoes are also used as raw materials for the pharmaceuticals, cosmetic industries, and food processing (Bhowmik et al. 2012). According to BPS (2020) data, the national production of tomato commodities from 2014 to 2018 has fluctuated; constraints on tomato cultivation caused the decline in tomato production in Indonesia. One is tomato disease, namely bacterial wilt caused by *Ralstonia solanacearum* (Smith 1896) Yabuuchi et al. 1996 (Seleim 2014; Kunwar 2020). The bacterial wilt of tomatoes causes enormous economic losses each year in many tropical and subtropical regions (Zheng et al. 2019). The disease causes a 35-95% decrease in tomato production (Singh et al. 2015). According to Singh et al. (2013), severe attacks due to tomato bacterial wilt disease can cause the death of cultivated plants and crop failure.

Bacterial wilting symptoms occur during the hottest part of the day and indicate that the youngest leaves are wilting. Only a few leaves will wilt at this stage, and if the temperature drops overnight, the plant will recover quickly. However, leaves remain green, leading to general wilting and yellowing of the leaves and eventual plant death (She et al. 2013). Another common symptom of bacterial wilting in the field is stunted crops. These symptoms can appear at any stage of plant development, but in the field, it is common for even healthy-looking plants to wilt suddenly during rapid fruit expansion (Singh 2017). The vascular bundles are affected in young tomato stems, and long narrow dark brown stripes become visible. Stem collapse can occur in young succulents from highly susceptible cultivars (Lu et al. 2018). However, plants may have symptomless infections under favorable conditions for a long time. In addition, after infection, pathogens can survive in infected plants and then spread from there.

Controlling tomato bacterial wilt has been challenging due to the wide host range, limited chemical control methods, long soil survival, and genetic diversity of *R. solanacearum* (Konappa et al. 2020). Until recently, controlling bacterial wilt is mostly done using chemical pesticides. Using chemical control methods for managing plant diseases has existed for several decades. However, excessive and repeated use of chemical pesticides, such as antibiotics and copper compounds, has resulted in a decline in helpful microorganisms, residual toxicity on food commodities, and bactericide-resistant strains (Le et al.

2020). Moreover, using chemical pesticides can cause several environmental problems that impact soil and water pollution (Ghorab and Khalil 2016), non-target organisms (Fishel 2019), and also humans, which impact farmers' and consumers' health (Sabarwal et al. 2018). Consequently, newer and better methods are needed to prevent bacterial wilt. In recent years, significant progress has been made in testing alternative control methods, particularly using microorganisms (biocontrol) for managing bacterial wilt (Mamphogoro et al. 2020). One of them is using the antagonist bacteria *Pseudomonas fluorescens* Migula 1895 P60 (Soesanto et al. 2021). The granule formulation of *P. fluorescens* is as effective as the granule bactericide in controlling the bacterial wilt of tomatoes (Soesanto et al. 2019). However, applying biocontrol agents in the field encounters several obstacles, such as ultraviolet exposure, environmental factors, and nutrient availability (Velivelli et al. 2014; Abd-Elgawad and Askary 2020). Therefore, secondary metabolites from *P. fluorescens* P60 are used to overcome these obstacles. On the other hand, *P. fluorescens* bacteria produce secondary metabolites such as antibiotics, volatile compounds, hydrogen cyanide (HCN), and phytohormones (Deveau et al. 2016). The biochemical properties of *P. fluorescens* include its ability to produce extracellular enzymes such as amylases, proteases, chitinases, cellulases, and gelatinases, bind Fe ions, generate Indole-3-acetic acid, and form antibiotics and siderophores (Deveau et al. 2016; Labhasetwar et al. 2019).

The secondary metabolite formula of *P. fluorescens* P60 was developed in liquid form (Soesanto et al. 2014). The obstacles encountered with liquid formulas are impractical to transport long distances, tend to evaporation, and limited storage time (Campos et al. 2016). *P. fluorescens* secondary metabolites have the potential to be tested in granular forms. Therefore, it is necessary to determine its shelf life, effect on bacterial wilting, and its performance on tomato plants.

MATERIALS AND METHODS

Exploration, virulence, and Koch postulates of *Ralstonia solanacearum*

Exploration

Tomato plants with symptoms of bacterial wilt were removed from the plantation, cut crosswise at the base of the stem, and put into a test tube filled with sterile water to observe the bacteria (oose) flow. Next, samples from the root were cut and washed with sterile water, and the epidermis was removed. Next, the roots were cut into smaller pieces with a size of 0.5 cm, and the surface was sterilized with 70% alcohol for 30 seconds and rinsed using sterile water for 1 minute, repeated twice. The sample is then put into 5 mL of sterile water for 1 minute to remove the bacterial mass (Rudrappa et al. 2018).

Virulence

The exploratory suspension containing bacteria was streaked with a loop needle on Triphenyl tetrazolium chloride (TZC) medium and incubated for 1x24 hours at room temperature of ±27°C (Zapata et al. 2020). Colonies of virulent *R. solanacearum* bacteria are characterized by white color, fluid shape, and pink colony center (Garcia et al. 2019). Bacterial colonies were transferred to a nutrient agar (NA) slanted medium.

Koch's postulates

The roots of the tomato seeds were cleaned and aseptically injured using a knife. The culture of *R. solanacearum* in a test tube aged two days was harvested using 10 mL of sterile water and then put into a new sterile test tube, the bacteria were taken using an ose needle, stirred, and serial dilutions were made using sterile water, so that the density and the volume became 10^7 cfu mL^{-1} and 50 mL, respectively. Tomato seedling roots were injured and then soaked in *R. solanacearum* suspension. The seedlings were then planted in polybags, and wilt symptoms were observed for two days after planting. The success of the Koch Postulates test is marked when the symptoms are the same as those observed in the field (Soesanto et al. 2019).

Preparation of secondary metabolites *Pseudomonas fluorescens* P60 granular formula

Pseudomonas fluorescens P60 was inoculated in 100 mL nutrient broth (NB) medium as much as 1 loop needle and shaken (Daiki orbital) for three days at 150 rpm at room temperature (26-28°C). The supernatant was prepared by centrifuging *P. fluorescens* P60 suspension (Sigma) at 3000 rpm for 20 minutes. The formed supernatant was taken, separated from the pellet (Soesanto et al. 2010), mixed with 100 mL of 20% xanthan gum in sterile distilled water, and allowed to stand for 20 minutes. Furthermore, as much as 500 g of sterile talcum powder carrier material was mixed and stirred until evenly distributed. The mixed formula is then granulated using a meat grinder (Fukuda) with a diameter of 5 mm and cut using scissors with 3-5 mm size. Finally, the formula was baked at 40^{O}C for 48 hours and stored at room temperature (±27°C) (Soesanto et al. 2019).

Pseudomonas fluorescens P60 secondary metabolite granular formula *in vitro* test

The *in vitro* testing phase was carried out by calculating the inhibition zone to test the shelf life using a Completely Randomized Design consisting of five treatments and four repetitions by putting the granular form on NA medium in Petri dishes with as many as four granules and incubating for one day (Soesanto et al. al. 2019). Next, 100 µL of the *R. solanacearum* bacterial suspension was taken and mixed with 4 mL of sterile 0.6% water agar, then poured into a Petri dish and incubated for 24 hours at room temperature. Granular form tests were carried out at 0, 2, 4, 6, and 8 weeks of storage by calculating the inhibition zone using the formula (Soesanto et al. 2019):

Inhibition diameter = Inhibition zone diameter of Granular Form – Granular Form diameter

Limited field test

The field test used a randomized block design with six treatments and four replications. The treatment consisted of plants inoculated only with *R. solanacearum* as the control, plants inoculated with *R. solanacearum* and added 1, 5, 10, or 15 g of granules plant^{-1}, and bactericide (20% agrimycin sulphate, 2 mL L^{-1}); so, there were 24 experimental units. Each experimental unit consisted of four tomato seedlings plant (var. Betavila, a 14-day-old seedling), so there were 96 plants. The application was carried out by burying the granules around the roots of the tomato seedlings to a depth of 7-10 cm, then planting them in polybags measuring 30 x 30 cm and put in an open space at ambient temperature (Soesanto et al. 2019). Inoculation of *R. solanacearum* was carried out by pouring the bacterial suspension (100 mL of a density of 10^7 cfu mL^{-1}) onto the roots without root injury on the third day after the application of the granular forms.

Observed variables

Pathosystem components

The incubation period was calculated from the time the seedlings were inoculated into plastic polybags containing a mixture of soil medium and manure until the first symptoms of the disease appeared. The units used are days after inoculation (DAI). Disease incidence was determined by calculating the percentage of wilted plants and total plants observed using the formula Ghosh and Mandal (2009), namely:

$$I(\%) = \frac{n}{N} \times 100\%$$

Where: I=Disease incidence; n=The number of affected plants; and N=The total number of sample plants observed.

AUDPC (Area Under Disease Progress Curve) was calculated using the formula Paraschivu et al. (2013), as follows:

$$AUDPC = \sum_{i}^{n-1} \left[\frac{y_i + y_{i+1}}{2}\right](t_{i+1} - t_i)$$

Where: AUDPC = Area under disease progress curve (%-weeks); Yi+1 = Observation data of the ith disease incidence + 1; Yi = Observation data of the 1st disease incidence; ti+1 = time of observation of the ith disease incidence +1; and ti=Observation time of 1st disease incidence.

Growth component

Plant height was observed at transplanting time and the last day of observation by calculating the difference in plant height. The fresh weight of shoots and roots was measured at the last observation. Fresh plant weight was weighed using a digital balance. The dry weight of shoots and roots was measured after drying in an oven at 60°C for two days or having a constant weight.

Qualitative phenol content test

The phenol content test was carried out qualitatively: saponins, tannins, and glycosides. The saponin test was carried out according to the modified methods of Fahrunnida and Pratiwi (2015). A total of 1 g of sample was ground using a mortar, added to 10 mL of distilled water, put into a test tube, and shaken, then one drop of 2 N HCl was added. The test tube was allowed to stand and observed for the presence or absence of stable foam. The sample contained saponins when a stable foam was formed with a 1-3 cm height for 30 seconds. The tannin test was carried out according to Simamora et al. (2021) modified. As much as 1 g of the sample was ground using a mortar, added 5 mL of 80% ethanol was then filtered. Next, 3 drops of 10% $FeCl_3$ were added to the filtered filtrate. The formation of dark blue or greenish-black color indicated the presence of tannins. The glycoside test was carried out according to Simamora et al. (2021) modified, too. As much as 1 g of the sample was ground using a mortar, then 5 mL of 80% ethanol was added, filtered, and dried over a water bath. After drying, the fat was removed by washing the hexane several times, so the pigment color was lost or the hexane solution was colorless. Next, the fat-free residue was heated over a water bath to remove the hexane. A 3 mL of $FeCl_3$ reagent was added, then stirred, and the mixture was transferred into a test tube, 1 mL of concentrated sulfuric acid solution was dripped through the test tube wall (be careful sulfuric acid was corrosive). The formation of blue or violet color indicates the presence of glycosides.

Data analysis

The F-test analyzed data at a 5% error rate. If the analysis results show significant and very significant differences, Duncan's Multiple Range Test (DMRT) at an error level of 5% was applied.

RESULTS AND DISCUSSION

Virulence and Koch's Postulates of *Ralstonia solanacearum*

The virulence of *R. solanacearum* bacteria can be determined by isolating it on a TZC medium. The isolation results of *R. solanacearum* obtained were according to Garcia et al. (2019), isolated virulent *R. solanacearum* bacteria colonies on TZC medium were characterized by a white color, a fluid shape, and a pink center of the colony (Figure 1.A). The virulence or malignancy of *R. solanacearum* is determined by the ability of the bacteria to produce exopolysaccharide compounds. The Koch's Postulates test results showed the same symptoms and signs in the field: the plants were wilting, and the leaves were drooping (Figure 1.B). This is following Chen et al. (2013), which stated that plants attacked by *R. solanacearum* experienced symptoms of drooping or wilted leaf stalks, and then eventually, the plants would die. Therefore, Koch's Postulate test was carried out to confirm the nature of pathogenic bacteria as disease-causing agents (Manganiello et al. 2021).

***In vitro* test of secondary metabolites of *Pseudomonas fluorescens* P60 granular formula**

Pseudomonas fluorescens P60 secondary metabolite granular form test produced an inhibition zone (Table 1). The results of the granular formula test showed significant differences between treatments. The 0-week shelf life had the largest inhibition zone. Therefore, the granular form is effective when used in a 4-week shelf life or could maintain a decrease in the inhibition zone of 21.67-23.34% compared to the control (0-week shelf life).

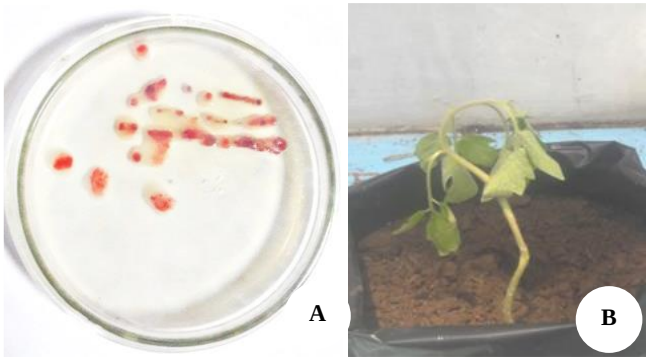

Figure 1. Virulent and Koch's Postulate test. Description: A. *R. solanacearum* colonies on TZC medium; B. Results of Koch's postulates

Table 1. The diameter of the inhibition zone from the secondary metabolite formulation of *P. fluorescens* P60 during the shelf life

Shelf life (WAS)	Inhibition zone diameter (mm)
0	15.00 a
2	11.75 b
4	11.50 b
6	8.00 c
8	3.25 d

Notes: Numbers followed by the same letters are not significantly different in the DMRT with an error rate of 5%; WAS=Weeks After Storage

According to Soesanto et al. (2011), *P. fluorescens* P60 produces secondary metabolites such as antibiotics and siderophore compounds, which act as fungistasis and bacteriostasis. The inhibition ability corresponds to the shelf life. The longer it is stored, the lower the inhibition effect. That follows Shrestha et al. (2016) opinion that the activity of antagonistic bacteria stored yearly against plant pathogens will decrease. A decrease in the ability of antagonistic bacteria is indicated by a decrease in their ability to produce secondary metabolites, which affect plant pathogens. Therefore, a decrease in antagonistic activity will result in no decrease in plant diseases. The decrease in the inhibition zone on the secondary metabolite test based on shelf life is thought to have occurred due to the low concentration of the secondary metabolite *P. fluorescens* P60 due to the decrease in the concentration of the bacteria. Storage within eight weeks and room temperature are thought to affect the concentration and effectiveness of the secondary metabolite of *P. fluorescens* P60.

According to Khameneh et al. (2019), the ability of an antimicrobial agent to inhibit microorganisms depends on the concentration and antimicrobial type produced. The higher the concentration of the antimicrobial agent, the larger the zone of inhibition formed. This is due to the higher concentration of antimicrobials, the more active substances contained in it, so the effectiveness in inhibiting bacteria will increase and produce a wider zone of inhibition. Conversely, at low concentrations, the antimicrobial content contained in an antimicrobial substance will be less; thus, its activity will decrease. Other factors that affect the decrease in the inhibition zone are thought to be temperature and storage (Campos et al. 2016). Storage carried out at room temperature allows contamination of the formula, thereby affecting the ability of the formula to inhibit bacterial growth (Morgan et al. 2006).

Application of *P. fluorescens* P60 secondary metabolite granular form against bacterial wilt of tomato

The analysis results between treatments and control did not show a significant difference in the incubation period of bacterial wilt (Table 2). However, in general, it can be seen that the application of *P. fluorescens* P60 secondary metabolites granular formula was able to affect *R. solanacearum*. That proved the secondary metabolite of *P. fluorescens* P60 in this form can play an active role in inhibiting the development of wilting pathogenic bacteria. Furthermore, applying these secondary metabolites with a more granular form tends to suppress the incubation period longer. This is presumably because the more secondary metabolites of *P. fluorescens* P60, the higher the antimicrobial concentration. According to Keswani et al. (2020), the higher the concentration of antimicrobial ingredients, the more active substances it contains, so the effectiveness in inhibiting bacteria will increase.

Disease incidence in the controls significantly differed between treatments (Table 2). The control and bactericidal treatments showed the highest disease incidence; bactericides have minimal effect on tomato wilt, it implies. Treatment of 15 g granular form of secondary metabolites *P. fluorescens* P60 resulted in the lowest incidence, reducing disease incidence by 75% compared to the controls. The ability of *P. fluorescens* P60 secondary metabolites in granular formulas to suppress disease incidence was consistent with in vitro testing. The inhibition zone in the test showed that the secondary metabolite of *P. fluorescens* P60 granular formula could inhibit the growth of the pathogen, thereby reducing the incidence of the disease. The secondary metabolites of *P. fluorescens* contain several antifungal and antibacterial compounds (Prabhukarthikeyan and Raguchander 2016).

Meanwhile, the AUDPC value for tomato bacterial wilt significantly differed between the control and treatment (Table 2, Figure 2). Applying 15 g of the secondary metabolite granular formula of *P. fluorescens* P60 reduced the AUDPC value by 74.7%. The high AUDPC value in the control treatment was due to the absence of the application of *P. fluorescens* P60 secondary metabolites or bactericides, which resulted in *R. solanacearum* being able to develop and infect plants without hindrance, causing the plants to wilt and die. Lower AUDPC scores are associated with lower disease progression and are consistent. The lower the AUDPC value, the lower the ability of the pathogen to develop and cause disease (Carisse 2016). Another factor that affected the high AUDPC value in the control treatment was due to the variety of tomato plants, which were reported to be susceptible to *R. solanacearum* (Wang et al. 2019). Susceptible varieties are more easily infected with *R. solanacearum*, resulting in high disease severity (Caldwell et al. 2017). This is consistent with the

higher incidence of bacterial wilt and the incubation period, which tends to be faster in the control treatment than the application of *P. fluorescens* P60 secondary metabolites granule and bactericidal formulas. The application of the bactericide has not been able to match the application of the 15 g granular formula of *P. fluorescens* P60 secondary metabolites, the severity of *R. solanacearum* was higher on plants treated with bactericide as compared to the 15 g granular application.

The plant height analysis showed significant differences (Table 3). The 5, 10, and 15 g granular formula treatments differed significantly from the controls. Treatment of 5, 10, and 15 g of *P. fluorescens* P60 secondary metabolite granular form could increase plant height compared to the control by 37.6, 35, and 44%, respectively. This is in line with the incidence of the disease that occurs. The less incidence of disease experienced by plants indicates the plant is healthier. Healthy plants grow better than plants diseased by bacterial wilt, increasing plant height. The increase in plant height is also thought to be due to the influence of the secondary metabolite of *P. fluorescens* P60. According to Soesanto et al. (2011) and Meliani et al. (2017), *P. fluorescens* can produce Indole-3-acetic acid (IAA), which can support plant growth. This is supported by Suresh et al. (2021) that *P. fluorescens*, in addition to producing IAA hormones, also produce various secondary metabolites, including siderophores and hydrogen cyanide (HCN), and shows potential efficacy not only in fighting phytopathogenic fungi but also in stimulating plant growth, so that plant growth will be accelerated.

Table 2. Effect of Treatment on pathosystem components

Treatments	Incubation period $(DAI)_{(tn)}$	Disease incidence (%)	AUDPC (%-weeks)
control	14.75 a	75.00 b	284.38 a
1 g	21.63 a	43.75 ab	187.50 b
5 g	24.63 a	25.00 a	134.38 c
10 g	23.25 a	25.00 a	103.13 c
15 g	25.44 a	18.75 a	71.88 d
bactericide	22.44 a	37.50 ab	162.50 b

Notes: Numbers followed by the same letter in the same column are not significantly different in the DMRT, with an error rate of 5%. Disease incidence data is transformed with $\sqrt{x + 0{,}5}$, DAI= days after inoculation; Bactericide = 20% agrimycin sulphate

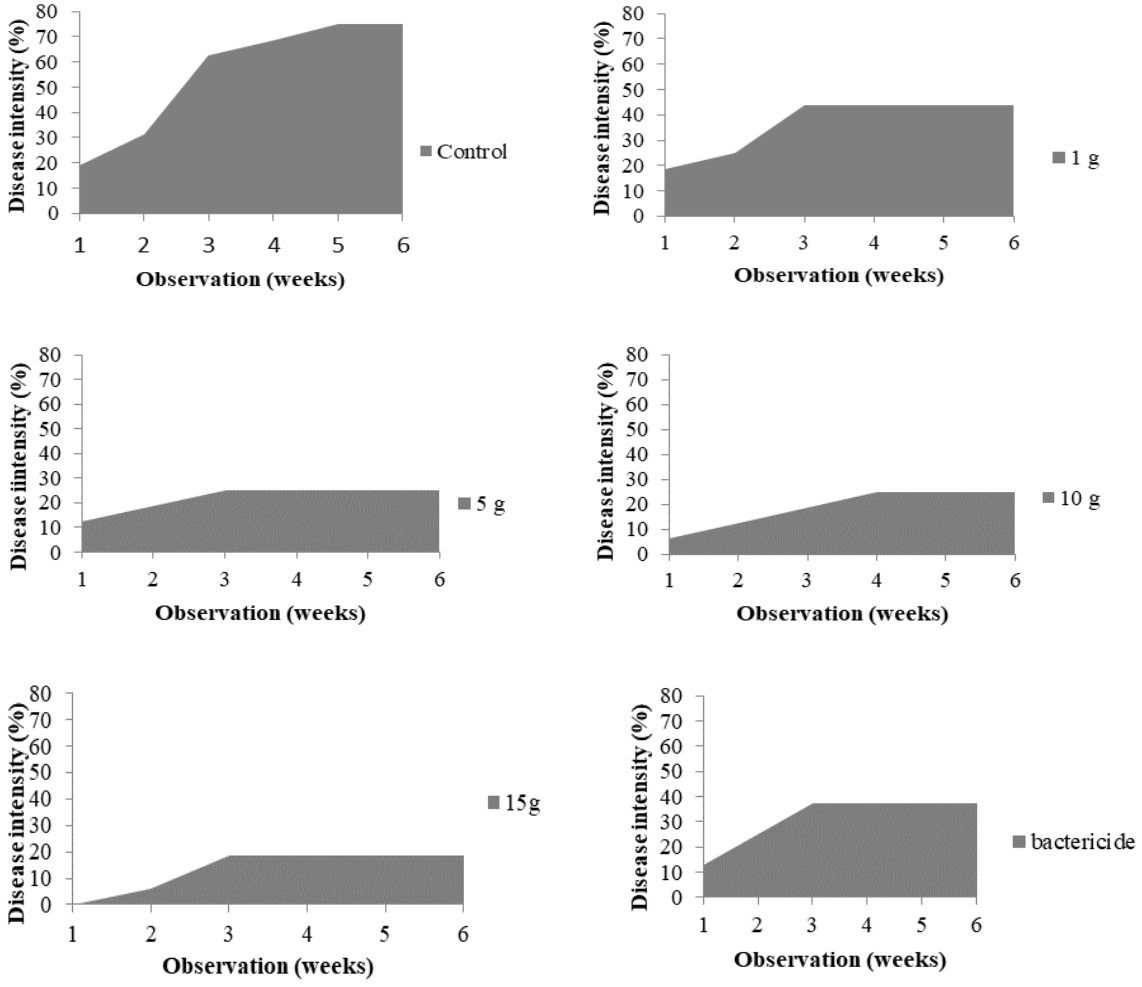

Figure 2. AUDPC value of tomato bacterial wilt

The canopy fresh weight analysis showed a significant difference (Table 3). The treatment of 10 and 15 g of secondary metabolite *P. fluorescens* P60 granular form significantly differed from the control, increasing fresh shoot weight by 61.72 and 65.65% compared to the control. The increase in crown weight is in line with the inhibition of the development of pathogenic bacteria so that the plants are healthier. In addition, the increase in plant fresh weight was in line with the presence of *P. fluorescens* P60 secondary metabolites, which produce IAA. Egamberdieva et al. (2017) explained that IAA could increase the growth of lateral roots and plant biomass.

The canopy dry weight showed no significant difference between the treatments and the control, although the control plants showed a lower crown dry weight (Table 3). That indicated the secondary metabolite of *P. fluorescences* P60 had not been able to increase the dry weight of plant shoots. However, there was a tendency to increase the dry weight of plant shoots in the treatment. Therefore, it is suspected that the nutrients from the growing media obtained by tomato plants have not met the required nutrient requirements. This follows the opinion of McDonald et al. (1996) that no significant difference in plant dry weight between treatments indicates that the nutrient requirement absorbed by the plant is insufficient to meet the plant's needs. Furthermore, Gerlin et al. (2021) and Kashyap et al. (2021) added that the physiological processes of tomato plants are hampered due to *R. solanacearum* developing in the xylem at the roots and base of the stem, then spreading to all parts of the plant and causing blockage of xylem by millions of *R. solanacearum* cells, resulting in the transport of water and nutrients from obstructed land.

The root dry weight analysis results showed a significant difference (Table 3). The treatment of 5, 10, and 15 g of *P. fluorescens* P60 secondary metabolites granular form was significantly different from the control and increased the root dry weight by 52.6; 59; and 62.5%, respectively, compared to the control. Soesanto et al. (2010) explained that applying *P. fluorescens* P60 supernatant at planting and 5 days after planting increased the dry weight of plant roots from 50.21 to 56.69%. Meanwhile, the root wet weight analysis results showed no differences between treatments (Table 3). However, the 5, 10, and 15 g of the granular formula increase the fresh weight of the roots. According to Soesanto et al. (2021), *P. fluorescens* P60 produces IAA, affecting root length, root surface area, and the number of root tips.

The results obtained from the phenol content test, which included saponins, tannins, and glycosides, qualitatively showed that the control treatment had the least phenol content compared to the treatments of the *P. fluorescens* P60 secondary metabolite granular formula and the bactericide (Table 4). The phenol content in the treatments was at least 1 g treatment. This is consistent with the high incidence of the disease and the fastest incubation period in the treatment of 1 g of *P. fluorescens* P60 secondary metabolite granular formula when compared to treatments of 5, 10, and 15 g of same and bactericide. The phenol content in the 5, 10, and 15 g treatment of *P. Fluorescens* P60 secondary metabolites granular formulations tended to be almost the same. The phenolic compounds' content indicates that the phenolic compounds produced affect the induced resistance of plants to pathogens (Babenko et al. 2019).

The high incidence of the disease and the fast incubation period of tomato plants due to attack by *R. Solanacearum* that causes bacterial wilt disease in the control are consistent with the low phenol content. Nicholson and Hammerschmidt (2003) and Misra et al. (2023) stated that phenolic compounds play a role in plant resistance to attack by pathogens. If the content of phenolic compounds is low, the plants are less resistant to pathogenic infections. The content of phenolic compounds in *P. fluorescens* P60 secondary metabolite granules varies according to disease severity and incubation period. The disease was less intense and had a longer latency when plants were treated with *P. fluorescens* P60 secondary metabolite granules compared to the controls of *P. fluorescens* P60 secondary metabolite granular formulation. This aligns with the phenolic content of the processed *P. fluorescens* P60 secondary metabolite granule formulations, with similar trends. Simamora et al. (2021) is *P. fluorescens* alone or combined with secondary metabolites from Trichoderma asperellum could increase the saponin, tannin, and glycoside content of the plant.

Table 3. Average plant height, shoot fresh weight, root fresh weight, shoot dry weight, tomato plant root dry weight due to treatment measured on the 28th days

Treatments	PH (cm)	FWS (g)	FWR (g)	DWS (g)	DWR (g)
Control	18.72 a	4.90 b	0.92 a	0.41 a	0.09 a
1 g	27.41 ab	7.16 ab	0.99 a	0.71 a	0.16 ab
5 g	30.00 b	7.08 ab	1.70 a	0.91 a	0.19 b
10 g	28.81 b	11.73 a	1.31 a	0.88 a	0.22 b
15 g	33.44 b	13.07 a	1.81 a	1.22 a	0.24 b
Bactericide	26.38 ab	7.58 ab	1.27 a	0.68 a	0.16 ab

Note: Numbers followed by the same letter in the same column are not significantly different in the DMRT, with an error rate of 5%. The data of crown fresh weight, root fresh weight, shoot dry weight, and root dry weight were transformed with √x. PH=plant height; FWS=Fresh Weight Of Shoots; FWR=Fresh Weight Of Roots; DWS=Dry Weight Of Shoots, DWR=Dry Weight Of Roots; Bactericide =agrimycin sulphate 20%

Table 4. Results of phenol analysis in each treatment qualitatively

Treatments	Saponins	Tannins	Glycosides	No. of analysis
control	+	+	+	3 +
1 g	++	+	+	4 +
5 g	++	++	++	6 +
10 g	++	+++	++	7 +
15 g	++	++	+++	7 +
bactericide	++	++	+	5 +

Note: a little (+), enough (++), a lot (+++) compared to control. Bactericide = agrimycin sulphate 20%

In conclusion, the *P. fluorescens* P60 secondary metabolites in granular formula showed good antagonistic activity up to the 4th week of storage and suppressed the inhibition zone between 21.6-23.3%. Treatment of 15 g of *P. fluorescens* P60 secondary metabolites granular formula reduced the incidence of tomato bacterial wilt by 75.0% and the AUDPC value by 74.7%. Treatment of 15 g of *P. fluorescens* P60 secondary metabolites granular formula increased plant height, root dry weight, and shoot fresh weight by 44, 62.5, and 65.6%, respectively. Treatment of *P. fluorescens* P60 secondary metabolites granular formula increased the content of phenolic compounds in tomato plants qualitatively.

REFERENCES

Abd-Elgawad MMM, Askary TH. 2020. Factors affecting success of biological agents used in controlling the plant-parasitic nematodes. Egypt J Biol Pest Control 30 (1): 1-11. DOI: 10.1186/s41938-020-00215-2.

Agrawal MS, Singh SK. 2009. Can tomato juice be used for prophylaxis in recurrent stone formers? Indian J Urol 25 (1): 149-150.

Babenko LM, Smirnov OE, Romanenko KO, Trunova OK, Kosakivska IV. 2019. Phenolic compounds in plants: biogenesis and functions. Ukr Biochem J 91 (3): 5-18. DOI: 10.15407/ubj91.03.005.

Bhowmik D, Kumar KPS, Paswan S, Srivastava S. 2012. Tomato-A natural medicine and its health benefits. J Pharmacog Phytochem 1 (1): 24-36.

BPS (Badan Pusat Statistik). 2020. Tabel dinamis produksi tanaman sayuran. https://www.bps.go.id/site/resultTab. [Indonesian]

Burton-Freeman B, Reimers KJ. 2011. Tomato consumption and health: Emerging benefits. Am J Lifestyle Med 5 (2): 182-191. DOI: 10.1177/1559827610387488.

Caldwell D, Kim BS, Iyer-Pascuzzi AS. 2017. *Ralstonia solanacearum* differentially colonizes roots of resistant and susceptible tomato plants. Phytopathology 107 (5): 528-536. DOI: 10.1094/PHYTO-09-16-0353-R.

Campos EVR, de Oliveira JL, Pascoli M, de Lima R, Fraceto LF. 2016. Neem oil and crop protection: From now to the future. Front Plant Sci 7: 1494. DOI: 10.3389/fpls.2016.01494.

Carisse O. 2016. Development of grape downy mildew (*Plasmopara viticola*) under northern viticulture conditions: Influence of fall disease incidence. Eur J Plant Pathol 144 (4): 773-783. DOI: 10.1007/s10658-015-0748-y.

Chen Y, Yan F, Chai Y, Liu H, Kolter R, Losick R, Guo J. 2013. Biocontrol of tomato wilt disease by *Bacillus subtilis* isolates from natural environments depends on conserved genes mediating biofilm formation. Environmental Microbiology 15 (3): 848-864. DOI: 10.1111/j.1462-2920.2012.02860.x.

Deveau A, Gross H, Palin B, Mehnaz S, Schnepf M, Leblond P, Dorrestein PC, Aigle B. 2016. Role of secondary metabolites in the interaction between *Pseudomonas fluorescens* and soil microorganisms under iron-limited conditions. FEMS Microbiol Ecol 92 (8): 1-11. DOI: 10.1093/femsec/fiw107.

Egamberdieva D, Wirth SJ, Alqarawi AA, Abd_Allah EF, Hashem A. 2017. Phytohormones and beneficial microbes: Essential components for plants to balance stress and fitness. Front Microbiol 8: 2104. DOI: 10.3389/fmicb.2017.02104.

Fahrunnida, Pratiwi R. 2015. Kandungan saponin buah, daun dan tangkai daun belimbing wuluh (*Averrhoa bilimbi* L.). Jurnal FKIP UNS 1 (1): 220-234. [Indonesian]

Fishel FM. 2019. Pesticide effects on non-target organisms. EDIS 2005 (14): 1-7. DOI: 10.32473/edis-pi122-2005.

Garcia RO, Kerns JP, Thiessen L. 2019. *Ralstonia solanacearum* species complex: a quick diagnostic guide. Plant Health Prog 20: 7-13. DOI: 10.1094/PHP-04-18-0015-DG.

Gerlin L, Escourrou A, Cassan C, Macia FM, Peeters N, Genin S, Baroukh C. 2021. Unravelling physiological signatures of tomato bacterial wilt and xylem metabolites exploited by *Ralstonia solanacearum*. Environ Microbiol 23 (10): 5962-5978. DOI: 10.1111/1462-2920.15535.

Ghorab MA, Khalil MS. 2016. The effect of pesticides pollution on our life and environment. J Pollut Effect Control 04 (02): 1-2. DOI: 10.4172/2375-4397.1000159.

Ghosh PP, Mandal NC. 2009. Some disease management practices for bacterial wilt of potato. J Plant Prot Sci 1 (1): 51-54.

Kashyap A, Planas-Marquès M, Capellades M, Valls M, Coll NS. 2021. Blocking intruders: inducible physico-chemical barriers against plant vascular wilt pathogens. J Exp Bot 72 (2): 184-198. DOI: 10.1093/jxb/eraa444.

Keswani C, Singh HB, García-Estrada C, Caradus J, He YW, Mezaache-Aichour S, Glare TR, Borriss R, Sansinenea E. 2020. Antimicrobial secondary metabolites from agriculturally important bacteria as next-generation pesticides. Appl Microbiol Biotechnol 104 (3): 1013-1034. DOI: 10.1007/s00253-019-10300-8.

Khameneh B, Iranshahy M, Soheili V, Bazzaz BSF. 2019. Review on plant antimicrobials: a mechanistic viewpoint. Antimicrob Resist Infect Control 8 (1): 1-28. DOI: 10.1186/s13756-019-0559-6.

Konappa N, Krishnamurthy S, Arakere UC, Chowdappa S, Ramachandrappa NS. 2020. Efficacy of indigenous plant growth promoting rhizobacteria and *Trichoderma* strains in eliciting resistance against bacterial wilt in a tomato. Egypt J Biol Pest Control 30: 106. DOI: 10.1186/s41938-020-00303-3.

Kunwar S, Bamazi B, Banito A, Carter M, Weinstein S, Steidl OR, Hayes MM, Allen C, Pare ML. 2020. First report of bacterial wilt disease of tomato, pepper and gboma caused by the *Ralstonia solanacearum* species complex in Togo. Plant Dis 105 (2): 484. DOI: 10.1094/PDIS-08-20-1665-PDN.

Labhasetwar AA, Bramhankar SB, Pillai TS, Isokar SS, Dinkwar GT, Bhure SS, Kharat VM. 2019. Biochemical and physiological characterizations of *Pseudomonas fluorescens*. Intl J Chem Stud 7 (1): 1785-1788.

Le KD, Kim J, Yu NH, Kim B, Lee CW, Kim JC. 2020. Biological control of tomato bacterial wilt, kimchi cabbage soft rot, and red pepper bacterial leaf spot using *Paenibacillus elgii* JCK-5075. Front Plant Sci 11:775. DOI: 10.3389/fpls.2020.00775.

Lu H, Lema A S, Planas-Marquès M, Alonso-Díaz A, Valls M, Coll NS. 2018. Type III secretion-dependent and -independent phenotypes caused by *Ralstonia solanacearum* in *Arabidopsis* roots. Mol Plant Microbe Interact 31 (1): 175-184. DOI: 10.1094/MPMI-05-17-0109-FI.

Mamphogoro TP, Babalola OO, Aiyegoro OA. 2020. Sustainable management strategies for bacterial wilt of sweet peppers (*Capsicum annuum*) and other Solanaceous crops. J Appl Microbiol 129: 496-508. DOI: 10.1111/jam.14653.

Manganiello G, Traversari S, Nesi B, Cacini S, Pane C. 2021. Rose: A new host plant of *Fusarium clavum* (*F. incarnatum-equiseti* species complex 5) causing brown spot of petals. Crop Protect 146: 105675. DOI: 10.1016/j.cropro.2021.105675.

McDonald AJS, Ericsson T, Larsson CM. 1996. Plant nutrition, dry matter gain and partitioning at the whole-plant level. J Exp Bot 47 (Special_Issue): 1245-1253. DOI: 10.1093/jxb/47.Special_Issue.1245.

Meliani A, Bensoltane A, Benidire L, Oufdou K. 2017. Plant growth-promotion and IAA secretion with *Pseudomonas fluorescens* and *Pseudomonas putida*. Res Rev J Bot Sci 6 (2): 16-24.

Misra D, Dutta W, Jha G, Ray P. 2023. Interactions and regulatory functions of phenolics in soil-plant-climate nexus. Agronomy 13 (2): 280. DOI: 10.3390/agronomy13020280.

Morgan CA, Herman N, White PA, Vesey G. 2006. Preservation of microorganisms by drying: A review. J Microbiol Method 66 (2): 183-193. DOI: 10.1016/j.mimet.2006.02.017.

Nicholson RL, Hammerschmidt R. 2003. Phenolic compounds and their role in disease resistance. Ann Rev Phytopathol 30 (1): 369-389. DOI: 10.1146/annurev.py.30.090192.002101.

Paraschivu M, Cotuna O, Paraschivu M. 2013. The use of the area under the disease progress curve (AUDPC) to assess the epidemics of *Septoria tritici* in winter wheat. Res J Agric Sci 45 (1): 193-201.

Prabhukarthikeyan SR, Raguchander T. 2016. Antifungal metabolites of *Pseudomonas fluorescens* against *Pythium aphanidermatum*. J Pure Appl Microbiol 10 (1): 579-584.

Rudrappa KB, Suryawanshi AP, Punitkumar ND, Ganesh JK, Lambani K, Singh R. 2018. Cultural and biochemical characterization of *Ralstonia*

solanacearum causing bacterial wilt in tomato. J Pure Appl Microbiol 10 (4): 3111-3115. DOI: 10.22207/JPAM.10.4.86.

Sabarwal A, Kumar K, Singh RP. 2018. Hazardous effects of chemical pesticides on human health-cancer and other associated disorders. Environ Toxicol Pharmacol 63: 103-114. DOI: 10.1016/j.etap.2018.08.018.

Seleim MAA, Abo-Elyousr KAM, Abd-El-Moneem KM, Saead FA. 2014. First report of bacterial wilt caused by *Ralstonia solanacearum* biovar 2 race 1 on tomato in Egypt. Plant Pathol J 30 (3): 299-303. DOI: 10.5423/PPJ.NT.10.2013.0101.

She XM, He ZF, Luo FF, Li HP. 2013. First report of bacterial wilt caused by *Ralstonia solanacearum* on *Ageratum conyzoi*des in China. Plant Dis 97 (3): 418. DOI: 10.1094/PDIS-08-12-0780-PDN.

Shrestha BK, Karki HS, Groth DE, Jungkhun N, Ham JH. 2016. Biological control activities of rice-associated *Bacillus* sp. strains against sheath blight and bacterial panicle blight of rice. PLoS One 11 (1): e0146764. DOI: 10.1371/journal.pone.0146764.

Simamora M, Basyuni M, Lisnawita. 2021. Potency of secondary metabolites of *Trichoderma asperellum* and *Pseudomonas fluorescens* in the growth of cocoa plants affected by vascular streak dieback. Biodiversitas 22 (5): 2542-2547. DOI: 10.13057/biodiv/d220511.

Singh D, Yadav DK, Sinha S, Choudhary G. 2013. Effect of temperature, cultivars, injury of root and inoculums load of *Ralstonia solanacearum* to cause bacterial wilt of tomato. Arch Phytopathol Plant Protect 47 (13): 1574-1583. DOI: 10.1080/03235408.2013.851332.

Singh S, Gautam RK, Singh DR, Sharma TVRS, Sakthivel K, Roy SD. 2015. Genetic approaches for mitigating losses caused by bacterial wilt of tomato in tropical islands. Eur J Plant Pathol 143: 205-221. DOI: 10.1007/s10658-015-0690-z.

Singh D. 2017. Bacterial wilt of solanaceous crops: Diagnosis,diversity and management. Indian Phytopath 70 (2): 151-163. DOI 10.24838/ip.2017.v70.i2.70607.

Soesanto L, Mugiastuti E, Rahayuniati RF. 2010. Kajian mekanisme antagonis *Pseudomonas fluorescens* P60 terhadap *Fusarium oxysporum* f. sp. *lycopersici* pada tanaman tomat *in vivo*. Jurnal Hama dan Penyakit Tumbuhan Tropika 10 (2): 108-115. DOI: 10.23960/j.hptt.210108-115. [Indonesian]

Soesanto L, Mugiastuti E, Khoeruriza. 2019. Granular formulation test of *Pseudomonas fluorescens* P60 for controling bacterial wilt (*Ralstonia solanacearum*) of tomato *in planta*. AGRIVITA J Agric Sci 41 (3): 513-523. DOI: 10.17503/agrivita.v41i3.2318.

Soesanto L, Mugiastuti E, Rahayuniati RF. 2011. Pemanfaatan beberapa kaldu hewan sebagai bahan formula cair *Pseudomonas fluorescens* P60 untuk mengendalikan *Sclerotium rolfsii* pada tanaman mentimun. Jurnal Perlindungan Tanaman Indonesia 17 (1): 7-17. [Indonesian]

Soesanto L, Mugiastuti E, Rahayuniati RF. 2014. Aplikasi formula cair *Pseudomonas fluorescens* P60 untuk menekan penyakit virus cabai merah. Jurnal Fitopatologi Indonesia 9 (6): 179-185. DOI: 10.14692/jfi.9.6.179. [Indonesian]

Soesanto L, Pradiptha CN, Mugiastuti E. 2021. Raw secondary metabolites of chitosan-enriched *Pseudomonas fluorescens* P60 to control corn sheath blight. Biosaintifika 13 (1): 113-120. DOI: 10.15294/biosaintifika.v13i1.28775.

Suresh P, Vellasamya S, Almaary KS, Dawoud TM, Elbadawi YB. 2021. *Fluorescent pseudomonads* (FPs) as a potential biocontrol and plant growth promoting agent associated with tomato rhizosphere. J King Saud Univ-Sci 33 (4): 101423. DOI: 10.1016/j.jksus.2021.101423.

Velivelli SLS, Vos PD, Kromann P, Declerck S, Prestwich BD. 2014. Biological control agents: from field to market, problems, and challenges. Trends Biotechnol 32 (10): 493-6. DOI: 10.1016/j.tibtech.2014.07.002.

Wang G, Kong J, Cui D, Zhao H, Niu Y, Xu M, Jiang G, Zhao Y, Wang W. 2019. Resistance against *Ralstonia solanacearum* in tomato depends on the methionine cycle and the γ-aminobutyric acid metabolic pathway. Plant J 97 (6): 1032-1047. DOI: 10.1111/tpj.14175.

Zapata LS, Álvarez JC, Romero-Tabarez M, Silby MW, Traxler MF, Behie SW, de Cassia Pessotti R, Villegas-Escobar V. 2020. Inducible antibacterial activity in the Bacillales by triphenyl tetrazolium chloride. Sci Rep 10 (1): 5563. DOI: 10.1038/s41598-020-62236-z.

Zheng X, Zhu Y, Wang J, Wang Z, Liu B. 2019. Combined use of a microbialrestoration substrate and avirulent *Ralstonia solanacearum* for the control of tomato bacterial wilt. Sci Rep 9 (1): 1-10. DOI: 10.1038/s41598-019-56572-y.