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Granular Formulation Test of *Pseudomonas fluorescens* P60 for Controling Bacterial Wilt (*Ralstonia solanacearum*) of Tomato *In Planta*

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ABSTRACT

Ralstonia solanacearum is the most devastating bacteria. *Pseudomonas fluorescens* P60 is a bacterial antagonist. This research aimed to study shelf life, antagonism and the effect of granular application of *P. fluorescens* P60 to control bacterial wilt and growth of tomato *in planta*. The research was conducted at the Plant Protection Laboratory and the screen house, Faculty of Agriculture, Jenderal Soedirman University, from October 2018 to March 2019. A randomized block design was used with six treatments and five replicates. The treatments were control, *R. solanacearum* + 1, 5, 10, and 15 g the granule, and bactericide (Agrimycine sulfate 20%). Variables observed were population density, clear zone, incubation period, disease incidence, disease intensity, area under disease progress curve (AUDPC), crop height, root length, crops fresh weights, and phenolic compound content qualitatively. Result showed that the formulation up to 10 weeks still performed a high *P. fluorescens* P60 population and good activity. All the granular and the bactericide effectively suppressed the disease indicated by the lenghtening incubation period of 22.77-26.25%, reducing the disease incidence as 60-85%, decreasing disease intensity as 65-85%, and decreasing AUDPC as 75.69-86.11%-days, increasing phenolic compound content qualitatively, and increasing crop height between 24.85-36.17%, and fresh weight between 46.04-57.13%.

INTRODUCTION

Tomato is one of important horticultural commodities in Indonesia (Sahera, Sabaruddin, & Safuan, 2012), Asia, and Africa; tomato is one of the most consumed vegetables in the world (Kim et al., 2016). According to the data from BPS (2018), Indonesian tomato production in 2013 was 992,780 tons, in 2014 was 916,001 tons, in 2015 was upto 877,801 tons, in 2016 amounting to 883,242 tons, and in 2017 was 962,845 tons. The decline in production from 2013-2015 could be caused by several factors. The factors affecting crop production are such as climatic features, human activities, soil condition, and disturbing organisme (plant pests and diseases) (Bebber, Ramotowski, & Gurr, 2013; Rengasamy, 2010).

Among the most devastating diseases attacking tomato is bacterial wilt caused by *Ralstonia solanacearum* (Smith) Yabucci et al. (Jiang et al., 2017; Nguyen & Ranamukhaarachchi, 2010). Among the plants host, this bacteria ranks among the most devastating pathogens worldwide especially in solanaceous crops such as chili, cotton, eggplant, tomatoes, tobacco, and potatoes (Lebeau et al., 2011), and many other plants such as bananas, sunflowers, Anthurium, peanut, rubber, sweet potatoes, ginger, cassava, turmeric, cloves, arrowroot, sesame, and patchouli (Nguyen & Ranamukhaarachchi, 2010; Tahat & Sijam, 2010). The bacterium penetrates through the root system and proliferates in xylem tissue. Irreversible wilting generally develops quickly, resulting in plant death (Lebeau et al., 2011).

In tomato, this disease is one of important diseases causing tomato wilt. The loss because of this disease is estimated to range from 1-5%. Evenmore the drastic loss due to this disease can reach 100%

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because of plants death (Aslam, Mukhtar, Hussain, & Raheel, 2017). To control this bacterial is difficult because of high variability, limited chemical control; highly survival in various environments and wide host range (Norman et al., 2009). The control of the bacterial wilt has been attempted through virulence strategies (Denancé et al., 2013; Jiang et al., 2014; Lebeau et al., 2011). The cultivar resistance of tomato plants to bacterial wilt can be overcome by newly emerging pathogenic bacterial strains. Resistant tomato germplasms, however, need more time and research to commercial production (Kim et al., 2016; Lee, Jang, Choi, Kim, & Choi, 2015).

The other controls of the disease have been carried out using chemical bactericides. The control in this way can cause problems, among others, agricultural product residues, environmental pollution, and causing negative impact on public health (Aktar, Sengupta, & Chowdhury, 2009; Bhardwaj & Sharma, 2013). Environmental friendly controls are recommended in order to ameliorate the adverse effects of commercial pesticides. Numerous biological control agents that are antagonistic to plant pathogen, such as *Pseudomonas* spp. strains, have been reported and applied as promising control alternatives and shown potential reduction to bacterial infection in plants (Al-Waily, Al-Saad, & Al-Dery, 2018; Couillerot, Prigent-Combaret, Caballero-Mellado, & Moënne-Loccoz, 2009).

Pseudomonas fluorescens has the ability to colonize roots and has antagonistic properties, namely antibiosis, competition, and inducing plant resistance so that plants are protected from plant pathogenic attacks (Couillerot, Prigent-Combaret, Caballero-Mellado, & Moënne-Loccoz, 2009; Seenivasan, David, Vivekanandan, & Samiyappan, 2012). *P. fluorescens* produces secondary antimicrobial metabolites, cyanide acid, and 2,4-diacetylphloroglucinol, phenazine, pyrrolnitrin, and pyoluteorin antibiotics (de Werra, Péchy-Tarr, Keel, & Maurhofer, 2009; Weller et al., 2012). Strain of *P. fluorescens* such as P60 is widely used to control plant pathogens (Soesanto, Mugiastuti, & Rahayuniati, 2010; 2011).

Formulation is the crucial issue for commercial products of bacterial agents or consortium of beneficial microorganisms (Bashan, De-Bashan, Prabhu, & Hernandez, 2014). On the other hand, formulation refers to the laboratory or industrial process of unifying the carrier with the bacterial strain. The antagonist *P. fluorescens* has been formulated in various ways due to easier and more practice in storage, transportation, and application. Formulation of *P. fluorescens* has been developed by using several materials such as liquid formula (Manikandan, Saravanakumar, Rajendran, Raguchander, & Samiyappan, 2010), manure formulas (Bashan, De-Bashan, Prabhu, & Hernandez, 2014), water in oil (Peeran et al., 2014), and pesta granulation (Caldwell, Hynes, Boyetchko, & Korber, 2012).

In addition, a granular formula needs to be developed in order to overcome some problems such as expensive production, poor storage ability, susceptible to environment condition, and efficacy application. The advantage of granular pesticides is that they are more economic, less harmful to beneficial insects such as bees, suitable for providing systemic influence, and to give a product which is safe and convenient for use (Gasic & Tanovic, 2013). The ideal conditions required for development of high efficiency formulations of biopesticides include selection of potent strains, shelf life, storage, application technology, quality control, biosafety, and registration (Keswani, Bisen, Singh, Sarma, & Singh, 2016).

The study was conducted to determine the shelf life and antagonism of *P. fluorescens* P60 granular formulation, the effect of *P. fluorescens* P60 granular formula application on the bacterial wilt, and on the growth of tomato plants *in planta*.

MATERIALS AND METHODS

Preparation of Bacterial Isolates

The research was conducted at the Plant Protection Laboratory and the screen house, Faculty of Agriculture, Universitas Jenderal Soedirman, from October 2018 to March 2019.

Isolate of *Ralstonia solanacearum*

Pathogenic bacteria *R. solanacearum* was obtained from the exploration of symptomatic withered bacteria. The bacteria are cultured on CPG TTC media until a single colony is obtained (Chaudhry & Rashid, 2011; Pontes, Fujinawa, & Oliveira, 2017). Hypersensitive test uses tobacco leaf (Balaž, Iličić, Maširević, Jošić, & Kojić, 2014). Pathogenicity of the *R. solanacearum* isolated pure culture was verified by Koch's postulates (Byrd & Segre, 2016).

P. fluorescens P60 Granular Formula

P. fluorescens P60 aseptically propagated on NA medium and incubated for 48 hours. The bacteria were then harvested and propagated with NB medium and shaken for 48 hours at 150 rpm A total of 150 ml of *P. fluorescens* P60 suspension of 10^{12} cfu/ml were mixed with 100 ml of 20% xanthan gum (Chantaro & Pongsawatmanit, 2010).

Storage Test of Formula *P. fluorescens* P60 Granules

The storage test of the granular formula was carried out for 12 weeks by calculating the population density of *P. fluorescens* P60 using the TPC (Total Plate Count) method (Brugger et al., 2012).

Antagonism Test of Granular Formula

The granular formula was placed on the NA medium and incubated one day. After incubation, 50 µl/ml *R. solanacearum* was poured into the dish and incubated for 24 hours at room temperature. The variable observed was the inhibition ability indicated by clear zone. Clear zones are measured by the formula (Gull & Hafeez, 2012):

Relative clear zone diameter = Clear zone diameter with colony – Colony diameter

In Planta Test

The granular formula was buried around tomato seedlings roots with a depth of 7-10 cm and then planted in polybags measuring 30 x 30 cm. Inoculation of *R. solanacearum* was carried out by spraying suspension (100 ml from density of 10^9 cfu/ml) to the roots on the third day after application of the formula (Manan, Mugiastuti, & Soesanto, 2018). The *in planta* test was carried out using a randomized block design (RBD) with six treatments and five replicates. The treatment consisted of control, and 1, 5, 10, and 15 g granules, and bactericide (Agrimycin sulfate 20%). The variables observed were incubation period, disease incidence, disease intensity, area under the disease progres curve (AUDPC), crop height, root length, weight of fresh tomato crops, and phenolic compound content qualitatively. Disease incidence was calculated by formula as follows (Hossain, Miah, & Bashar, 2011):

Percent disease incidence = (Total number infected plants/Total number of plants) x 100%

Disease intensity (IP) was calculated using the formula:

$$IP = (\sum(n \times v)) / (Z \times N) \times 100\% \text{ ....1)}$$

where:
n = number of leaves in each attack category
v = scale value from each category
Z = the highest attack scale category value
N = number of leaves observed

The attack category was based on Manan, Mugiastuti, & Soesanto (2018) as follows:
0 = no attack / health
1 = over reach 25%
2 = negligence reaches 50%
3 = overdue to reach 75%
4 = wandering reaches all plants

The value of AUDPC was calculated by a formula (Paraschivu, Cotuna, & Paraschivu, 2013):

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

where:
Yi + 1 = i + 1 observation data
Yi = i observation data
ti + 1 = i + 1 observation time
ti = i observation time
n = total number of observations.

Analysis of phenolic compounds qualitatively was carried out at the end of the research according to Altemimi, Lakhssassi, Baharlouei, Watson, & Lightfoot (2017).

Data Analysis

The data of *P. fluorescens* P60 granular effect on incubation periode, disease incidence, disease intensif, crop height, root length, and crop fresh weight were analyzed by the F test at the error level of 5% and continue with Duncan Multiple Range Test (DMRT) (< 0.05) when significant results occurred. The AUDPC was analyzed descriptively.

Table 1. Density of *P. fluorescens* P60 and clear zone diameter during storage

Storage time (weeks)	Population of *P. fluorescens* P60 (cfu/g)	Average of clear zone diameter (mm)
0	2.50×10^{11}	No tested
2	3.12×10^{14}	No tested
4	2.44×10^{14}	No tested
6	1.85×10^{16}	14.25
8	1.13×10^{16}	12.25
10	4.30×10^{15}	11.50

RESULTS AND DISCUSSION

The Influence of *P. fluorescens* P60 on Population Density and Clear Zone Diameter

The population of *P. fluorescens* P60 bacteria at the time of making granules was 2.5 x 10^{11} cfu/g (Table 1). During storage it increased until the 8th week and decreased by the 10th week. This is related to the availability of nutrients. Allegedly nutrients were obtained from xanthan gum and talcum powder as ingredients.

Xanthan gum is thought to be a source of glucose for growth. Xanthan gum is produced through fermentation of organic matter, such as dextrose, sucrose, and flour by *Xanthomonas campestris* (Chantaro & Pongsawatmanit, 2010). The persist and increased of *P. fluorescens* P60 population were also influenced by the carrier material used. Tripathi, Das, Chandra, & Varma (2015) mentioned that the nature of stable carrier materials were able to maintain the stability of the formula against various treatments and storage time so that nutrients for bacteria remained available. Asadi, Soltani, Mohammadi, Khodadadi, & Abdollahy (2019) stated that talcum powder ($Mg_3SiO_{10}(OH)_2$) has soft and high stability properties. The antagonism test results between *P. fluorescens* P60 and *R. solanacearum* granular formulas showed clear zones. This shows that *P. fluorescens* P60 activity can continue. The clear zone formed shows that *P. fluorescens* P60 is capable of producing antibiotics to inhibit the growth of *R. solanacearum*. Couillerot, Prigent-Combaret, Caballero-Mellado, & Moënne-Loccoz (2009) and Pathma, Rahul, Kennedy, Subashri, & Sakthivel (2011) stated that *P. fluorescens* produced secondary metabolites such as antimicrobial, cyanide acid, and 2,4-diacetylphloroglucinol, phenazine antibiotic, pyrolnitrin, pyoluteorin. The diameter of the clear zone granular formula from the 6th week to the 10th week decreased. The decreasing diameter of the clear zone was consistent with the decrease in the population of *P. fluorescens* P60 bacteria during the storage period. The decreased bacterial population caused the number of antibiotics produced to decrease. The ability of bacteria to inhibit growth can be indicated by the size of the clear zone diameter that appears. The results showed that the diameter of the clear zone was between 11.50 – 14.25 mm. The diameter size according to Pavithra, Sathish, & Ananda (2012) includes a strong category. Based on the calculation of the density of *P. fluorescens* P60 granular formula and antagonism test up to week 10, the granular formula still had good antagonism activity and high *P. fluorescens* P60 population.

The Effect of *P. fluorescens* P60 Granular Formulae on Pathosystem Components

Pathosystem components of the bacterial wilt were affected by the application of *P. fluorescnes* P60 in granular formulation significantly indicated by lengthening the incubation period and decreasing the disease incidence, the disease intensity, and AUDPC as well as phenolic compound content qualitatively.

The statistical analysis of the incubation period (Table 2) showed a significant difference between the treatment of *P. fluorescens* P60 and bactericidal with a control treatment. The fastest incubation period was the control treatment of 30.35 dai (day after inoculation). Grain treatments of 1, 5, 10, and 15 g, and bactericide were able to suppress the incubation period of 24.51; 22.77; 26.25; 23.07; and 25.61%, respectively. These results are in line with the *in vitro* testing of the granular formula. The increasing population and the appearance of clear zones show that the granular formula is able to inhibit pathogen growth, so the incubation period is longer than the control. The delay of the incubation period of granular and bactericidal treatments was not significantly different based on statistical tests. This is presumably because of the secondary metabolites produced. *P. fluorescens* P60 bacteria can produce secondary metabolites capable of controlling pathogens in the form of protease, siderophore, and antibiotics (Couillerot, Prigent-Combaret, Caballero-Mellado, & Moënne-Loccoz, 2009; Pathma, Rahul, Kennedy, Subashri, & Sakthivel, 2011). *P. fluorescens* has also been reported to produce antibiotics such as 2,4-diacetylphloroglucinol phenazine, pyrolnitrin, oomycin, pyoluteorin, furanomycine, tensin, tropolone (Couillerot, Prigent-Combaret, Caballero-Mellado, & Moënne-Loccoz, 2009; Gupta, Parihar, Ahirwar, Snehi, & Singh, 2015; Sivasakthi, Usharani, & Saranraj, 2014; Trippe, McPhail, Armstrong, Azevedo, & Banowetz, 2013). The treatment of *P. fluorescens* P60 and bactericidal granules was not significantly different based on the statistical tests. This shows that *P. fluorescens* P60 has capabilities comparable to bactericide. Based on these results, it is expected that *P. fluorescens* P60 granules can be used as a biopesticide to replace bactericide, so controlling bacterial wilt becomes environmentally friendly.

The statistical analysis of the incidence and intensity of the disease (Table 2) shows a significant difference between the treatment of *P. fluorescens* P60 and bactericidal with control. This is in line with the incubation period and *in vitro* test. The highest incidence and intensity of the disease is the control treatment of 100%. Grain treatments of 1, 5, 10, and 15 g, and bactericides have disease incidence and intensity between 15–40%. Based on the level of suppression of the disease, the treatment was able to reduce the incidence of disease by 60, 75, 80, 85, and 75%, respectively. Disease intensity could be reduced by each treatment as 65, 75, 80, 85 and 75%, respectively. The statistical analysis also showed no difference between the treatment of *P. fluorescens* P60 granular formula of 1, 5, 10, and 15 g, and bactericide. Although it did not show a difference but in general, the more granular formulas added tend to reduce the incidence and intensity of the disease (Table 2). It is suspected that the bacterial population of *R. solanacearum* was applied to the treatment of *P. fluorescens* P60 and bactericidal granules decreased. Bashan, De-Bashan, Prabhu, & Hernandez (2014) reported that the population of *R. solanacearum* at the highest control was compared to the number of populations in the treatment of the organic liquid formula *P. fluorescens* P60. The treatment of *P. fluorescens* P60 granules of 1, 5, 10, and 15 g had lower incidence and disease intensity values than controls. Emphasis on the incidence and intensity of the disease was thought to be determined by the initial population of bacteria in the granular formula when applied to plants. The initial population is 1.85 x 10^{16} cfu/g granules which was 6 weeks at a shelf life. The population was able to suppress the incidence and intensity of the disease by 60% and 65%. The results of the study are in line with the research of Manan, Mugiastuti, & Soesanto (2018), the treatment of *P. fluorescens* P8 and P16 sprayed as much as 50 ml with a density of 2 x 10^{11} cfu/ml and repeated 4 times could reduce the incidence and intensity of the disease by 65.95 and 31.57%, respectively. The treatment of *P. fluorescens* P60 in an organic liquid formula could reduce the disease incidence by 100% (Manan, Mugiastuti, & Soesanto, 2018). The high incidence and intensity of the disease were related to the activity of secondary metabolites produced by *P. fluorescens* P60. Gupta, Parihar, Ahirwar, Snehi, & Singh (2015) explained that siderophore is able to bind iron elements around plants, so pathogens lack iron elements for growth. Antibiotics produced by the bacteria genus *Pseudomonas* are reported to be able to control plant pathogens. These antibiotics include amphisin, 2,4-diacetylphloroglucinol, oomycin A, phenazine, pyoluteorin, pyrrolnitrin, tensin, tropolone, and cyclic lipopeptides, and cyanide acid.

The highest calculation results for the AUDPC value were the control treatment. Grain treatments of 1, 5, 10, and 15 g and bactericide had lower AUDPC values than the control treatments (Fig. 1). Decreasing the value of AUDPC for each treatment is 75.69; 78,47; 86.11; 86.11; and 81.25%, respectively. The low value of AUDPC is proportional to the low progression of the disease. The lower the value of AUDPC, the lower the ability of pathogens to develop and to cause disease will be (Carisse, 2016). Control has the highest incidence and intensity of disease and the fastest incubation period compared to treatment. This is because pathogens develop well due to the absence of *P. fluorescens* P60 or bactericides around plant roots. The rapid development of pathogens results in faster symptoms of bacterial wilting, high incidence and intensity of disease. Treatment with *P. fluorescens* P60 has a lower AUDPC value and is in line with the lower incidence and intensity of disease and a longer incubation period than controls.

Table 2. The effect of *P. fluorescens* P60 granular formula on incubation period, disease incidents, disease intensity, and AUDPC

Treatments	Incubation period (dai)	Disease incidents (%)	Disease intensity (%)	AUDPC (%-days)
Control	30.35 b	100 a	100 a	630.00
1 g granule	40.20 a	40 b	35 b	153.13
5 g granule	39.30 a	25 b	25 b	135.63
10 g granule	41.15 a	20 b	20 b	87.50
15 g granule	39.45 a	15 b	15 b	87.50
Bactericide	40.80 a	25 b	25 b	118.13

Remarks: Numbers followed by the same letters in the same column show no significant difference in DMRT (< 0.05), dai = days after inoculation

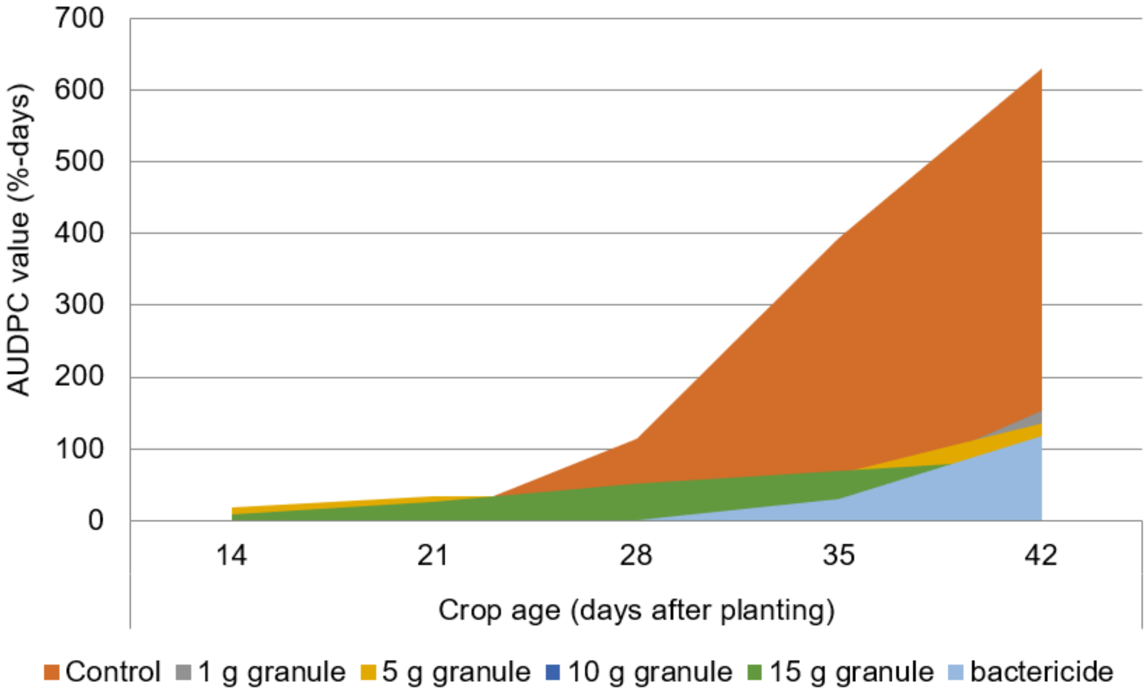

Fig. 1. AUDPC value of bacterial wilt in tomato influenced by *P. fluorescens* P60 granular formula

The results of saponins, tannins, and glycosides analysis qualitatively indicates that the treatment increases the phenol compound (Table 3). In general, the value of phenolic compounds increases due to treatment in values that vary from a little to many. Allegedly this shows that the treatment provides induced resistance to plants.

Table 3. The effect of *P. fluorescens* P60 granular formula on phenolic compound content qualitatively

Treatments	Saponin	Tannin	Glycosides
Control	+	-	+
1 g granule	++	+++	+++
5 g granule	+++	++	+++
10 g granule	++++	++	++
15 g granule	++++	+	+++
Bactericide	++++	+++	+++

Remarks: Signs (-) and (+) indicate the presence or absence of phenol compounds; (-) = none, (+) = little, (++) = rather a lot, (+++) = quite a lot, (++++) = lots

Induced resistance is defined as natural stimulation of plants to control pathogens (De Vleesschauwer & Höfte, 2009). Plant-induced resistance can be determined by analyzing the content of phenol compounds, such as saponins, tannins, and glycosides in plant tissues. The more phenol compounds, the higher resistance of plants will be (Eyles, Bonello, Ganley, & Mohammed, 2010). The results of the research by Li et al. (2013) showed that phenol compounds produced by plants were negatively correlated with the intensity of root diseases. This shows that an increase in phenol compounds will further suppress the intensity and incidence of disease. The content of phenol compounds is related to the incubation period, the incidence and intensity of the disease. Based on the statistical tests, the variable was not significantly different between the treatment of *P. fluorescens* P60 and bactericides in harmony with the test results of phenol compounds between treatments. This shows that *P. fluorescens* P60 granules can be an alternative to control the bacterial wilt and minimize the use of chemical pesticides.

The Effect of *P. fluorescens* P60 Granular Formula on Growth Components

The application of *P. fluoresens* P60 granular formula can increase crop height and crop fresh weights of tomato but do not influence tomato root length (Table 4).

Table 4. The effect of *P. fluorescens* P60 granular formula on crop height, root length, and crop fresh weight of tomato

Treatments	CH (cm)	RL (cm)	CFW (g)
Control	61.32 b	21.59 a	29.00 b
1 g granule	96.07 a	21.22 a	67.65 a
5 g granule	88.95 a	22.59 a	65.39 a
10 g granule	90.82 a	22.20 a	64.98 a
15 g granule	81.60 a	19.22 a	55.64 a
Bactericide	87.22 a	18.35 a	53.74 a

Remarks: Numbers followed by the same letters in the same column show no significant difference in DMRT with an error rate of 5%; CH = crop height, RL = root length, CFW = crop fresh weight

The statistical analysis of plant height and fresh weight of plants (Table 4) shows that there are differences in plant height and fresh weight of plants between controls and treatment of *P. fluorescens* P60 and bactericidal. The control treatment has the lowest plant height and fresh weight of the plant. This is thought to occur because the wilting bacteria damaged the xylem transport network of plants so that transportation experienced interference. The disrupted nutrient transportation causes plants not to grow properly. According to Zuluaga, Puigvert, & Valls (2013), *R. solanacearum* enters into plant tissue through wounds on the roots. The attack of *R. solanacearum* on the xylem transport network causes brown color in the xylem transport vessels of plants. *R. solanacearum* damages the cell walls of plant tissue due to the production of pectinesterase, cellulase, and protease enzymes. This resulted the transportation being disrupted (Lowe-Power, Khokhani, & Allen, 2018). The applications of *P. fluorescens* P60 as much as 1, 5, 10, and 15 g, and bactericides were able to increase the height and fresh weight of plants. The increase in plant height is 36.17; 31.06; 32.48; 24.85; and 29.69%, respectively, and the fresh weight of each plant is 57.13; 55.65; 55,37; 47.88; and 46.04%, respectively. This is in line with the incidence and intensity of the disease. The fewer occurrences and intensity of the disease, the healthier the plants will be. Healthy plants will grow and develop well so that the plant height and fresh weight of the plant will increase. The increase in plant height and fresh weight of plants was thought to be related to secondary metabolites produced by *P. fluorescens* P60. Gupta, Parihar, Ahirwar, Snehi, & Singh (2015) reported that Plant Gowth Promoting Rhizobacteria (PGPR) like the genus *Pseudomonas* also produced cytokinins and giberellins as growth hormones. Druege, Franken, & Hajirezaei (2016) suggested that the IAA hormone can increase cell elongation in a vertical direction, thus spurring the growth of plant height. In addition, the IAA hormone will increase the elasticity of the cell wall and water will enter easily so that the cell will grow. The enlarged cells cause plant weight to increase. Cytokinin hormones cause the elongation of the stem by stimulating cell division and elongation, so that higher plants can be obtained.

The statistical analysis of root length (Table 4) shows no differences in root length between controls, *P. fluorescens* P60 granular treatment, and bactericides. This shows that the treatment of *P. fluorescens* P60 and bactericide is not able to increase root length. Besides the presence of hormones produced by the plants themselves, the ability of PGPR *P. fluorescens* P60 applied to plants is thought to have not been optimum in spurring root length increments. Therefore, the root length is relatively the same for both the control and treatment of *P. fluorescens* P60 and bactericidal granules. According to Soesanto, Mugiastuti, & Rahayuniati (2010), the application of PGPR to the field is not always optimum, requiring time to adapt and spur plant growth. Although biological agents make plants are able to survive from the attack of pathogens but their growth cannot be supported because the production of secondary metabolites including the nature of PGPR which plays a role for plant growth is not optimum.

CONCLUSION

The formula for *P. fluorescens* P60 until the 10th week still had good antagonistic activity and a high *P. fluorescens* P60 population. Treatment of *P fluorescens* P60 1, 5, 10, and 15 g, and bactericides could reduce the development of bacterial wilt, which was able to delay the incubation period between 22.77-26.25%, reducing the incidence of disease between 60-85%, reducing the disease intensity between 65-85% and decreasing the value of AUDPC between 75.69-86.11% compared to the controls. The treatment of *P. fluorescens* P60 and bactericidal granules provided induced resistance which indicated by an increase in phenol compounds in the form of saponins, tannins and glycosides compared to the controls. The treatment of *P. fluorescens* P60 and bactericidal granules was able to increase plant height between 24.85–36.17% and the fresh weight of plants between 46.04–57.13%.

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