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Research article

Application of Secondary Metabolites of Two Pseudomonas fluorescens Isolates to Control Bacterial Wilt of Potato

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Abstract.
The focus of this research was to determine how secondary metabolites from two isolates of *Pseudomonas fluorescens* (P8 and P60) affected potato plant resistance to bacterial wilt (*Ralstonia solanacearum*) as well as potato growth and productivity. For four months, this experiment was carried out at Serang Village's potato field, in Karangreja District, Purbalingga Regency, at an altitude of 1285 m above sea level, with Andisol soils, an average temperature of 22.31^{o}C, and relative humidity of 84.09%. A randomized block design was used with a control treatment, drenching with 1.5 g/L bactericide (20% streptomicin sulphate) administered six times, and drenching with secondary metabolites of *P. fluorescens* P8 or *P. fluorescens* P60 administered three, six, nine, and 12 times. Each treatment was carried out five times. Each treatment resulted in a different incubation period, disease intensity, infection rate, plant height, number of tubers, tuber weight per plant, wet and dry weight of crop, fresh and dry weight of root, number of branches, and phenolic compound analysis. The results showed that applying *P. fluorescens* P8 and *P. fluorescens* P60 secondary metabolites to potato plants can induce resistance by increasing the content of phenolic compounds in the plants. Drenching with secondary metabolites from *P. fluorescens* P8 or *P. fluorescens* P60 12 times can reduce the incubation period by 9.23%, the intensity of disease by 75%, and the incidence of disease by 53.57%. Plant height, crop dry weight, root fresh weight, root dry weight, number of tubers, and tuber weight per plant can all be increased by using secondary metabolites.

Keywords: bacterial wilt, potato, Pseudomonas fluorescens, secondary metabolites.

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1. Introduction

Potato is the world's largest non-cereal food crop and the world's fourth largest food crop after rice, wheat and corn. Potato plays an important role as a supporter of diversification programs in order to achieve sustainable food security [1]. Potato production in Indonesia fluctuated from 2015 which was recorded at 1,219,270 tons to 1,314,657 tons in 2019 [2]. This productivity is still relatively low, when compared to potato production in temperate countries which can reach 370 million tons [3].

One of the constraints in potato production is bacterial wilt disease caused by *Ralstonia solanacearum* (E.F. Smith) [4], [5], [6]. The direct loss of yield by *R. solanacearum* varies according to the host, the crop, the climate, the type of soil, the sowing method and the strain, and the loss of yield of potatoes is between 33-90% [7]. Chemical control is almost impossible to apply, and antibiotics such as, ampicillin, tetracycline, streptomycin, and penicillin have almost no effect [8]. The extensive use of synthetic fungicides can have negative effects [9], [10]. Alternative controls to reduce these effects are effective but environmentally friendly, that is, the use of biopesticides based on antagonistic microorganisms, such as *Pseudomonas fluorescens*. [11].

Based on the research results [12], *P. fluorescens* P8 can inhibit the growth of *R. solanacearum* and *Meloidogyne* sp, and can inhibit the growth of *F. oxysporum* in vitro by 34.78%. The application of *P. fluorescens* P60 in the liquid formula of golden snail broth was able to reducing the incidence of bacterial wilt of potato plants ranging from 47.8-60.9% when compared to controls, but only 26.44% when compared to the use of bactericides [13]. In addition, there was a tendency that the effect of *P. fluorescens* P60 1, 3, and 5 times of drenching can enhanced the yield weight of potato tubers by 35.57%, 34.96%, and 28.53%, respectively.

Based on the above, it is necessary to conduct research with the aim of knowing the effect of secondary metabolites of *P. fluorescens* P8 and *P. fluorescens* P60 on the effect of potato plant resistance against bacterial wilt, and potato's growth and yield.

2. Methodology

This research was conducted at the potato farmers' land in Serang Village, Karangreja District, Purbalingga Regency (altitude ± 1,285 m above sea level, average temperature 22.310C, humidity 84.09%, and andisol soil type), for 4 months.

2.1. Preparation of P. fluorescens P8 and P. fluorescens P60 and R. solanacearum

Propagation of *P. fluorescens* P8 and P60 was carried out with King's B medium by taking the bacterial colonies with an ose needle, then diluted and incubated for 2 x 24 hours so that they were ready for use. *R. solanacearum* was propagated using Nutrient Broth media, shake with a Daiki Orbital Shaker at 150 rpm for 3 days in room temperature, and then the density was calculated.

2.2. Preparation of secondary metabolites P. fluorescens P8 and P. fluorescens P60

The secondary metabolites of antagonistic bacteria were prepared using gold snail broth. The golden snail meat is washed with clean water, after being washed it is weighed as much as 400 g for 1 liter of water, boiled until cooked and added 2 g of shrimp paste per 1 liter of water [13]. Once cooked, the broth is filtered through sterile cotton and placed in a sterile drum under heating, sealed and cooled. Next, each antagonist bacteria was put into each sterile jerry can, shaken with a Daiki Orbital Shaker at 150 rpm for 3 days in room temperature, then the density was calculated.

2.3. Calculation of the initial population of P. fluorescens P8 and P60 and R. solanacearum

The populations of *P. fluorescens* P8 and *P. fluorescens* P60 were calculated by graded dilution, then grown on solid King's B medium for 24 hours, until the density was 10^{12} cfu per mL. The population of *R. solanacearum* was calculated by graded dilution and grown on NB medium for 48 hours, until the density was 10^{8} cfu per mL.

2.4. Land and potato seeds preparation

The land was cleared of previous crop residues and hoeed to form beds measuring 70 x 100 cm, height 30 cm, distance between beds 40 cm, drainage was made about 50 cm deep and 50 cm wide. Potato seeds used were seeds of the Granola G3 variety. Seedlings were obtained from Certified Seedling Bina Seed, Bandung Regency. The number of seeds needed for this research is 300 potato seeds.

2.5. Potato planting

The potato tubers were planted in the morning with 30 cm × 70 cm plant spacing, 30 cm plot spacing. Previously, the seedlings were soaked according to each treatment using water, *P. fluorescens* P8 and *P. fluorescens* P60 with a density of 1.8 x 10^{12} upk/mL and bactericide for 15 minutes. At the time of planting, 630 g of Chicken manure fertilizer was also applied per planting hole, 4.2 g of urea, 5.2 g of SP36 and 6.3 g of KCl between the planting holes. Potato seeds were planted one tuber per planting hole, the tubers were placed horizontally with the shoots facing up, 8-10 cm deep, and then covered with soil.

2.6. Application of secondary metabolites P. fluorescens P8 and P. fluorescens P60

The secondary metabolites derived from *P. fluorescens* P8 and *P. fluorescens* P60 were given in two ways of application, namely soaking the seeds for 15 minutes before planting, and drenching directly around the plant roots 14 days after planting with a dose of 50 mL/plant for 1-6 applications. While 100 ml/plant for 7-12 applications at intervals of three days. The population density of *P. fluorescens* P8 and *P. fluorescens* P60 used for producing the secondary metabolites was 1.8 x 10^{12} cfu/mL.

2.7. Application of R. solanacearum

R. solanacearum was drenched as much as 20 ml per planting hole before the potato seeds were planted and aged 14 days after planting (dap). The density of *R. solanacearum* used was 1.9 x 10^{8} cfu/ml.

2.8. Maintenance

Plant maintenance was done by watering the plants when it was not raining, and cleaning weeds is done twice at the age of 3 and 6 weeks. In addition, a fungicide with active ingredients of 75% chlorotalonil and 80% mankozeb was sprayed to control *Phytophthora infestans*.

2.9. Harvest

Harvesting was done when the potato plants are 80 days after planting in the morning when the weather is bright and dry. One of the characteristics of potato plants that have been recommended for harvesting is when 80% of the potato plants have turned yellow.

2.10. Experimental design

The research design was prepared using Randomized Block Design (RBD) with six treatments, that is, control treatment, bactericide (a.i. streptomycin sulfate 20%) as much as 1.5 g/L for 6 times, drenching the secondary metabolites for 3, 6, 9, and 12 times and

replicates five times for each treatment. Each research unit consisted of 10 plants so a total of 300 experimental plants used.

2.11. Observed variables

The variables observed are the incubation period, intensity of the disease, incidence, infection rate, final density of pathogens, increase in plant height, number of branches, fresh weight of the crown and dry weight, fresh weight of the root and dry weight, number of tubers per plant, maximum volume of tubers, weight of each tuber and tissue analysis. The calculation of the intensity of bacterial wilt was carried out using the formula [14]: I = Σ (ni x vi)/(Z x N), where I = attack intensity (%), ni = number of plants on the 1st scale, vi = scale value for each 1st plant, N = number of plants observed, and Z = highest scale value. The damage score (score) is based on a scale for bacterial wilt of potato plants [15], *i.e.*, 0 = 0% no leaf wilt, 1 = 1-25% leaf wilt, 2 = 26-50% leaf wilt , 3 = 51-75% of leaves withered, and 4 = >75% of all leaves withered or died. Disease incidence was calculated based on [16], with the formula DI = (n/N) x 100%, where DI = disease incidence (%), n = number of affected plants, and N = number of observed plants. The infection rate is calculated based on the formula according to [17], namely: r = 2,3/t (log 1/1-Xt–log 1/1-X0), where r = infection rate, X0 = proportion of disease beginning. Xt. = proportion of disease at time t, and t = observation time. The method of calculating the final density of *Ralstonia solanacearum* is to take 1 g of soil sample, then suspend it evenly in 9 ml of sterile water, and dilute it to 108. The suspension from the last dilution of 1 ml was put into solid NA media and leveled with L glass. Observations and density calculations were carried out after 48 hours.

2.12. Data Analysis

The data obtained is analyzed by analysis of variance of the F test to produce the effect of the treatment being tried. If there is a significant difference, it is continued with the Honest Significant Difference (HSD) test with significant level of 5%.

3. Result and Discussion

3.1. The effect of Treatment on Pathosystem Components

The results of statistical analysis of the effect of treatment on the components of the pathosystem are presented in Table 1.

3.2. Incubation period

The statistical analysis result showed that the application of secondary metabolites has a significant impact on the incubation period of bacterial wilt. (Table 1). This proves that the secondary metabolites of *P. fluorescens* P8 and *P. fluorescens* P60 can slow down the incubation period of bacterial wilt.

Table 1: Effect of treatment on the average incubation period, morbidity, intensity, infection rate, and pathogen density in later stages.

Treatments	Incubation period (dai)	Disease intensity (%)	Disease incidence (%)	Infection rate (unit/days)	Pathogen late density (cfu/g)
K0	59.74 a	26 a	56 a	0.0027 a	7.8×10^{11}
K1	62.46 ab	11.5 bc	38 ab	0.0009 b	6×10^{9}
K2	60.11 ab	17.5 ab	46 ab	0.0013 bc	1.2×10^{11}
K3	62.84 ab	15.5 abc	40 ab	0.0012 bc	1.1×10^{11}
K4	62.85 ab	9.5 bc	32 ab	0.0008 bc	8×10^{7}
K5	65.82 b	6.5 c	26 b	0.0003 c	6×10^{7}

Note: Numbers followed by different letters in the same colomn show a significant difference in the BNJ test with an error rate of 5%. K0 = control, K1 = bactericide with active ingredient streptomycin sulfate 20% for 6 times, K2-K5 = drenching of secondary metabolites of *P. fluorescens* P8 and *P. fluorescens* P60 for 3, 6, 9, and 12 times, respectively; dai = days after inoculation.

The lowest incubation period was shown by the application of secondary metabolites of *P. fluorescens* P8 and *P. fluorescens* P60 for 12 times, i.e., 6.08 dai or experiencing a delay of 9.23% compared to the control. Delay the emergence of disease symptoms in the secondary metabolites consistent with the disease intensity, disease incidence, and infection rate was higher than the late pathogen population in the growing media (Table 1), and content of tissue analysis of phenolic compounds qualitatively (Table 2). This is thought to be due to the ability of the secondary metabolites so that it can be a inhibitor for pathogens in attacking plants. Besides that there is a difference in the final density of the pathogen which causes the secondary metabolites to effectively delay the incubation period of bacterial wilt disease in potato plants. This is in accordance with the opinion of [18] and [19] that treatment with *P. fluorescens* was able to produce

secondary metabolites including the antibiotic 2.4-diacetylphloroglucinol which is able to affect plant resistance by increasing phenols in plants.

3.3. Disease Intensity

The treatment had a very significant effect on disease intencity between the treatment and control (Table 1). This proves that secondary metabolite can control bacterial wilt. The lowest disease intensity was shown by application of the secondary metabolites of *P. fluorescens* P8 and P60 for 12 times which is 75% compared to control when compared with bactericide by 43.47%. Low disease intensity is in line with the low variable incubation period, infection rate, late population of the pathogen in the growing media (Table 1) and content of tissue analysis of phenolic compounds qualitatively (Table 2). This is presumably because application of the secondary metabolites is able to inhibit and kill the bacteria pathogen. The ability of the secondarfy metabolites to reduce disease intensity was also not statistically significant compared with the treatment of bactericide so that it is hoped that in the future it can replace the use of synthetic bactericides which have quite a lot of negative effects on the environment. This is in line with the opinion of [20] that *P. fluorescens* with its secondary metabolites can inhibit the growth of pathogens and reduce the presence or severity of disease through some mechanisms and induced resistance by increasing the phenol content in the plant resulting in decrease the intensity of disease than control. This is appropriate with [19] that the secondary metabolites of *P. fluorescens* especially the siderophores are mainly responsible for the antagonistic activity of the pathogens.

3.4. Disease incidence

The secondary metabolite application influenced significantly the incidence of disease compared to control in (Table 1). This proves that secondary metabolite can control bacterial wilt. The lowest incidence of disease was found in the application of *P. fluorescens* P8 and *P. fluorescens* P60 secondary metabolites for 12 times. This application can suppress the disease incidence by 53.57% compared to control and when compared to bactericide by 31.57%. The ability of secondary metabolites to reduce the incidence of bacterial wilt disease is related to the variables of incubation period, intensity of incidence, infection rate, and subsequent population density of pathogenic bacteria (Table 1) and the content of tissue analysis of phenolic compounds qualitatively (Table 2). This is presumably because it is related to the various mechanisms contented in the

secondary metabolites. *P. fluorescens* is reported to be capable of producing secondary metabolites including siderophores, pterin, pyrrole, and phenazine. Siderophores can act as fungistatic and bacteriostatic and proteolytic enzymes (which may be involved in parasitism) [21] and affect plant systemic resistance [22].

3.5. Infection rate

The rate of disease infection was significantly different between the secondary metabolites and the control. (Table 1). This proves that the secondary metabolites of *P. fluorescens* P8 and P60 can reduce the rate of infection of bacterial wilt. The lowest infection rate was found in the secondary metabolites of *P. fluorescens* P8 and *P. fluorescens* P60 for 12 times of 0.0003 units/day and the highest infection rate was found in the control of 0.0027 units/day. The low rate of infection in the secondary metabolites was in line with the disease intensity, incubation period, disease incidence, and the late population of the pathogen (Table 3) and the content of tissue analysis of phenolic compounds qualitatively (Table 4). This proves that the secondary metabolites can act as pathogen competitors so as to reduce the rate of infection of bacterial wilt disease. Suppression and inhibition of the intensity of bacterial wilt is thought to be due to parasitism involving the secondary metabolites. This is in line with [21] and [23] that the secondary metabolites produced by *P. fluorescens* contain antimicrobial traits and lytic enzymes such as protease, amylase, pectinase, chitinase, gelatinase, phosphate solubilization, siderophore, hydrogen cyanide, cellulase, and Indole acetic acid (IAA).

3.6. Late pathogen density

The late population density of the pathogen showed differences in each treatment compared to control and bactericide (Table 1). The late stage density of pathogenic Ralstonia solanacearum was the highest among the controls, and the secondary metabolite drenching for 12 times was the lowest. This result is consistent with the high intensity, incidence, infection rate, and a delay in the incubation period disease (Table 1). This is presumably because in the control there were no inhibition activity by component of the secondary metabolites and *R. solanacearum* can multiply well in the local environment which is also supported by environmental conditions suitable for the development of the pathogen in the experimental field, namely air temperature, humidity, and soil pH, respectively, of 20.75-24.52°C, 80.71-86%, and 6-6.5, respectively, so that the pathogen can infect potato plant roots. This is in accordance with the opinion of [24] and [25] that

the development of the life cycle of *Ralstonia solanacearum* is greatly affected by environmental factors such as temperature, humidity of the air and water, and plant fitness factors that influence the development of the pathogen *R. solanacearum* to grow rapidly at air temperature conditions of 24-35°C but its development decreases at temperatures above 35°C or below 16°C.

3.7. The effect of Treatment on Popato Plant Resistance

Based on the results of tissue analysis qualitatively in potato plants different results were obtained in each treatment (Table 2). The overall results of the three tests of phenolic compounds it appears that the secondary metabolites of *P. fluorescens* P8 and *P. fluorescens* P60 can increase the phenolic compounds content in potato plants. This is in line with the disease intensity, disease incidence, infection rate, delay the incubation period, and the late density of pathogens (Table 1). This is presumably because the administration of antagonists can affect plant resistance to the pathogen *R. solanacearum*. This is in accordance with the opinion according to [26] that chemical resistance is indicated by the formation of chemical compounds capable of preventing the growth and development of pathogens. The compounds in question are secondary metabolites, among others: alkaloid compounds, phenol, flavonides, glycosides,and phytoalexins so that plants are protected from disease infection and have resistance due to attack by pathogens that can cause damage to plants [27].

Table 2: Effect of treatment on the content of phenol compounds qualitatively.

Treatments	Saponin	Glycoside	Tannin
K0	++	+	+
K1	+	++	++
K2	++	++	++
K3	++	++	++
K4	++	++	+++
K5	+++	+++	+++

Not: - = no phenol, + = little phenol, ++ = enough phenol, and +++ = a lot of phenol. K0 = control, K1 = K1 = bactericide with active ingredient streptomycin sulfate 20% 6 times, K2-K5 = drenching of secondary metabolites of *P. fluorescens* P8 and *P. fluorescens* P60 for 3, 6, 9, and 12 times, respectively; dai = days after inoculation.

This is reinforced by the statement of [28] that some phenolic compounds in plants can poison pathogens. Besides that. this is in accordance with the opinion of [29] said that phenolic compounds are compounds found in various types of higher plants

and have physiological effects. Among them are compounds resulting from secondary metabolism that can affect plant resistance.

3.8. Treatment Effect on Growth and Yield of Potatoes

The results of statistical analysis of the components of growth and yield of potato plants are presented in Table 3.

Table 3: Effect of treatment on growth and yield components.

Treatments	Plant height increase (cm)	Fresh weight of crown (g)	Dry weight of crown (g)	Freash weight of roots (g)	Dry weight of roots (g)	Number of tubers per plant	Tuber weight (g/plant)
K0	20.98 a	72.66 a	7.94 a	43.42 a	5.94 a	17.24 a	429.12 a
K1	23.91 ab	89.74 c	8.31 ab	46.64 a	6.15 a	17.54 a	472.42 a
K2	27.98 bc	79.12 bc	8.99 ab	46.30 a	6.94 ab	17.90 a	470.54 a
K3	25.67 abc	76.28 ab	8.58 ab	43.44 a	7.09 ab	17.16 a	483.32 a
K4	27.10 bc	91.04 c	9.11 b	52.56 b	8.12 bc	18.24 a	527.98 b
K5	27.92 c	91.58 c	9.15 b	53.64 b	9.53 c	19.26 b	525.86 b

Note: Numbers followed by different letters in the same variable show a significant difference in the BNJ test with an error rate of 5%. K0 = control, K1 = bactericide with active ingredient streptomycin sulfate 20% for 6 times, K2-K5 = drenching of secondary metabolites of *P. fluorescens* P8 and *P. fluorescens* P60 for 3, 6, 9, and 12 times, respectively; dai = days after inoculation.

3.9. Plant height increase

The increase in plant height was significantly different in each treatment compared to the control (Table 3). The lowest plant height gain was found in the control while the highest increase in plant height was found in the drenching application of secondary metabolites derived from *P. fluorescencs* P8 and *P. fluorescens* P60 for 12 times, i.e., 24.86% compared to control. This is consistent with other variables (Table 3) and disease intensity, disease incidence, and infection rate (Table 1). This is presumably because the secondary metabolites suppress pathogens development and stimulate plant growth so that the ability of plants to carry out photosynthesis will run well followed by a rapid rate of growth and elongation of cells and tissue formation, hence stem growth, leaves and roots will run fast too. This is in accordance with the research results of [30], [31], and [32] that the application of *P. fluorescens* P60 can reduce plant pathogens in the soil and increase the growth of the tested plants.

3.10. Fresh and dry weight of plant crown

The results of the statistical analysis of the fresh weight and the dry weight of the plant crowns gave significant different from the control. (Table 3). The positive effect on fresh weight and dry weight of the highest plant crown found in the treatment of *P. fluorescens* P8 and *P. fluorescens* P60 secondary metabolites, each increased by 20.65. and 13.22 % compared to control. The high fresh weight and dry weight of the crown was related with the low variable of disease intensity, disease incidence, and infection rate (Table 1) and the high variable of plant height increase, fresh and dry weight of roots, number of tubers, and tuber weight per plant (Table 3). This is presumably because the secondary metabolites can inhibit the development of pathogens, increase plant-induced resistance, and stimulate plant growth so that the physiological processes are not disturbed and growth and yield of potato plants will grow and develop optimally. This is in accordance with the opinion of [33] and [20] that *P. fluorescens* in addition to being able to suppress pathogens can also produce growth hormones that can stimulate plant root growth and act as Plant Growth Promotion Rhizhobacteria (PGPR) including auxins, gibberellins, and cytokinins. This is reinforced by the results of research according to [31] that *P. fluorescens* P60 can enhance the wet weight of shallot plants by 51.40 %.

3.11. Fresh and dry weight of roots

The statistical analysis result of root fresh weight showed that there was no significant difference from the control (Table 3). However, there was a tendency to have a positive effect on the highest root fresh weight in the secondary metabolites of *P. fluorescens* P8 and *P. fluorescens* P60 drencehd for 12 times of 53.64 g or able to increase 19.05% compared to control. The positive effect on the highest root fresh weight was thought to be related to the variable of plant height increase, fresh and dry weight of plant crown, number of tubers, and yield of tuber weight per plant (Table 3) and low disease intensity, disease incidence, and infection rate (Table 1). This is in accordance with the opinion of [34] which states that root development is strongly influenced by genes but these genes then interact with the environment. Soil environmental factors that affect root development either directly or indirectly are moisture, temperature, nutrient content, toxic material, biological agent, and the state of the soil (strength and volume of soil) [35]. Therefore, if one of the factors does not support the root development will adjust and be uniform.

The statistical analysis of dry weight of the root were significantly different than the control (Table 3). The lowest of dry weight of root was 5.94 g in control while the drenching application of the secondary metabolites of *P. fluorescencs* P8 and *P. fluorescens* P60 for 12 times of 9.53 g showed the highest root dry weight by 37.67% than control. Dry weight of root in the treatment was thought to be related to the low disease intensity, disease incidence, and infection rate (Table 1) and the high variable of plant height increase, dry and fresh weight of plant crown, dry and fresh weight of roots, number of tubers, and weight of tuber per plant (Table 3). This is presumably because the secondary metabolites functions as a biological control agent and the antagonist bacteria tested were also PGPR. This is consistent with [36] that its ability to produce Indole Acetic Acid (IAA) can be used as the main criterion in selecting PGPR because IAA will affect, root surface area length of root, and root tips number.

3.12. Tubers number and weight of tuber per plant

Results of statistical analysis of the tubers number and weight of tuber per plant were significantly different from control (Table 3). Positive effect on increasing the tubers number per plant and weight of tuber per plant was shown in secondary metabolites application of *P. fluorescens* P8 and *P. fluorescens* P60 for 12 times, each able to increase by 10.48. 0.76. and 18.39% compared to control. The tuber weight per plant was found in the secondary metabolite treatment which is in line with the high variable of plant height increase, dry and fresh weight of plant crown, dry and fresh weight of roots, and tubers number (Table 3) and low disease intensity, disease incidence, and infection rate (Table 1). This is presumably because the secondary metabolites can inhibit the development of pathogens and stimulates plant growth and is also able to increase plant-induced resistance compared to control that are susceptible to pathogens so that the number and weight of tubers produced is high. This is in accordance with the opinion of [37] and [38] that the antagonist role *of P. fluorescens* P60 in addition to producing antibiotics also capable as PGPR.

4. Conclusion

Application of secondary metabolites P. fluorescens P8 and P. fluorescens P60 can affect potato plant resistance by increasing the content of phenolic compounds. The treatment of soaking the seeds for 15 minutes. and watering of secondary metabolites P. fluorescens P8 and P. fluorescens P60 12 times. able to prolong the incubation period

9.23%. reduce disease intensity by 75%. reduce the incidence of disease by 53.57%. reduce the infection rate of 88.88%. increase the final density of antagonist 1.8 x 1015 upk/g soil. reduce the final pathogen density 6 x 107 upk/g soil. increased plant height increase by 24.86%. increased root dry weight 37.67%. and increased tuber/plant weight by 18.39%.

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