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Research Article
Applications of *Pseudomnas fluorescens* P60 in Controlling Basal Stem Rot (*Sclerotium rolfsii* Sacc.) on Dragon Fruit Seedlings

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Abstract

Background and Objective: At the beginning of planting dragon fruit, seedlings often have decay at the base of the stem caused by the fungus *Sclerotium rolfsii* (*S. rolfsii*) Sacc. This disease is very detrimental for dragon fruit farmers. An alternative control of this disease may also use biological antagonistic microbes. The aim of this research was to evaluate *Pseudomonas fluorescens* (*P. fluorescens*) P60 against *Sclerotium rolfsii* in dragon fruit seedlings and its plant growth-promoting ability. **Methodology:** Experiments were conducted for the dragon fruit plantations of Agrowisata Kusumo Wanadri at Glagah Village, Temon Subdistrict, KulonProgo Regency. There were five treatments which includes: 1: Untreated control, 2: *P. fluorescens* P60 as a drench, 3: *P. fluorescens* P60 as a soak, 4: *P. fluorescens* P60 as a soak and drench and 5: Benomyl as a soak and drench. There were six replicates of each treatment and arranged in a randomized complete block design. The soak and drench were repeated six times, each for 10 min, 30 days after planting. Following the treatments, the following parameters were evaluated such as disease incidence, late populations of *S. rolfsii*, plant fresh weight, root length, number of shoots, the shoot length and qualitative phenolic compound contents. Data were analyzed by F-test to determine the effect of treatment. **Results:** This study results showed that the use of *P. fluorescens* P60 effectively suppress stem rot caused by *S. rolfsii* on dragon fruit seedlings, by inhibiting the incubation period as 29.76%, lower the disease intensity as 66.65%, suppress the disease incidence of 46.14%, lower late sclerotia population of 74.79% and increase the content of phenolic compounds qualitatively. The application of *P. fluorescens* P60 was most appropriate to the nursery dragon fruit cuttings material by soaking in a solution of *P. fluorescens* P60 for 10 min. **Conclusion:** The use of *P. fluorescens* P60 can promote the growth of dragon fruit seedlings, covering the difference in seedlings fresh weight of 52.87%, root length as 40.09%, number of shoots as 34.76% and long shoots as 8.99%.

Key words: Basal stem rot, dragon fruit, seedlings, *Pseudomonas fluorescens* P60, *Sclerotium rolfsii*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Dragon fruit is grown increasingly in Indonesia. The local market is currently flooded with export products. Dragon fruit contains many nutrients and give certain health benefits[1]. Hence, dragon fruit was cultivated in many areas, thus encouraging the rapid development of dragon fruit.

The successful cultivation of dragon fruit was through good preparation and quality of seedlings. Seedlings have to be healthy, vigor and free of pests and diseases was some of the characteristics of high-quality seeds[2]. At the beginning of planting dragon fruit, seedlings often have decay at the base of the stem which was caused by the fungus *Sclerotium rolfsii* Sacc. This disease is very detrimental for dragon fruit farmers[3]. *S. rolfsi* can be controlled through several ways such as by application of fungicide, soil solarization and crop rotation[4]. Other alternative control of this disease may also use biological antagonistic microbes[5].

Antagonistic *P. fluorescens* P60 capable of inhibiting the formation of new mikroskleretium *Verticilium dahlia* on a test plant *Arabidopsis thaliana* and eggplant[6,7]. The biological agents have been used to control fungal diseases caused by *S. rolfsii* in peanuts[8]. However, the antagonistic bacteria have not been tried for *S. rolfsii* on dragon fruit. The purpose of this study was to determine (1) The effectiveness of the use of *P. fluorescens* P60 in the control of *S. rolfsii* on dragon fruit seedlings, (2) The most appropriate way to apply the *P. fluorescens* P60 on dragon fruit seedlings and (3) The effect on the growth of seedlings dragon fruit plants.

MATERIALS AND METHODS

Research conducted at the dragon fruit plantation land belonging to Agro Kusumo Wanadri, located in the village Glagah, Temon, Kulon Progo, Yogyakarta, in 2014, with altitude ±10 m above sea level and the type of soil regosol.

Propagation and inoculation fungal pathogens: *S. rolfsii* obtained from nurseries land of dragon fruits in Agro Kusumo Wanadri, cultured using PDA media. The media were poured in petri dishes approximately 20 mL/petri. The media were waited until cool, then be inoculated with the mycelium orsclerotia of *S. rolfsii*, then incubated for 5-7 days at room temperature. The pathogenic fungi inoculation was performed using fungal sclerotia of *S. rolfsii* sclerotia as much as 5 sclerotia per planting hole before planting.

Preparation of plant growing media: Planting seedlings of dragon fruit used polybag measuring 25×20 cm filled with soil and cow manure in the ratio 1: 1.

Preparation of stem cuttings: Stems of dragon fruit were quite old to be cut with the size of 20-30 cm length and made tapered at the base.

Treatments: Random block design (RBD) were used and the treatments tested were control (with pathogen inoculation), pathogen inoculation, cutting material soaked with *P. fluorescens* P60 for 10 min, inoculation of pathogens, *P. fluorescens* P60 medium drench on crops, inoculation pathogens, cutting material soaked with *P. fluorescens* P60 for 10 min and drenching on the planting medium and pathogen inoculation, seeds soaked with a solution of benomyl for 10 min and drenching the planting medium. Treatments were repeated six times consisted of five seedlings for each unit. *P. fluorescens* P60 solution was used with concentrations of 10 mL L^{-1}.

Planting and maintenance: Stem cuttings were planted in polybags, with a depth of approximately 5 cm. Maintenance performed includes weeding and watering.

Observations

Patosistem components: The main component was the intensity of the disease observed using the following Eq. with modified category[9]:

$$IP\ (\%) = \frac{\Sigma(n \times v)}{Z \times N} \times 100$$

Where:
IP = Intensity disease (%)
n = Number of samples are diseased
v = Value score illness
Z = Highest score
N = Number of samples was observed

With a category 0 = no symptoms, 1 = symptoms stem yellow 2 = Symptoms of stem rot 0-25%, 3 = Symptoms of stem rot 26-50%, 4 = Symptoms of stem rot 51-75%, 5 = Symptoms of stem rot>75%. In addition, also observed the incubation period, incidence of the disease and the final sclerotia population.

Components of growth: Shoot length, root length and the difference in plant fresh weight.

Tissue analysis: Plant tissue analysis qualitatively was observed to test the content of phenolic compounds (saponins, tannins and glycosides)[10] modified.

Statistical analysis: Data were analyzed by F-test to determine the effect of treatment. Significant difference was followed by DMRT with the error rate of 5%.

RESULTS AND DISCUSSION

Effect of treaments on the pathosystem component: Results of variance analysis showed that *P. fluorescens* P60 and fungicides benomil significantly affect the incubation period of the disease (Table 1). The fastest incubation period was found on control, with pathogen inoculation and the slowest incubation period was occurred in the treatment of soaked for 10 min and drench on the planting medium, or may inhibit the incubation period.

Effects of treaments on pathosystem component

Incubation period: Results of variance analysis showed that *P. fluorescens* P60 and fungicides benomil significantly affect the incubation period of the disease (Table 1). The fastest incubation period was found on control, with pathogen inoculation and the slowest incubation period was occurred in the treatment of soaked for 10 min and poured on the planting medium, or may inhibit the incubation period. The delay the incubation period because of the competition between pathogen and antagonist, so it took longer on pathogen to infect seedlings[11].

Treatment with *P. fluorescens* P60 could inhibit the growth of fungi *S. rolfsii* indicated by a longer incubation period than the fungicide. It was said that *P. fluorescens* P60 could prolong the incubation period of the disease. *P. fluorescens* has the characteristic of "plant growth promoting rhizobacteria" (PGPR), produces antibiotics and siderophores, able to colonize plant roots, as well as impacting on plant resistance[12,13].

Disease intensity: *P. fluorescens* P60 significant affected the disease intensity of dragon fruit seedlings (Table 1). Dragon fruit seedlings treated with *P. fluorescens* P60 had lower disease intensity when compared to control and fungicide benomyl. This research was supported by Soesanto *et al.*[14] and Alabouvette *et al.*[15] which states that the pathogen is difficult to penetrate the root system colonized by antagonist. Competition might also occur for colonization of the root surface and plant tissues[16].

Treatment of *P. fluorescens* P60 by soaking could reduce the intensity of the biggest diseases, namely 66.65% and drenching application reduced the intensity of the disease by 60%. However, if the application of *P. fluorescens* P60 done multiple, which was soaked and watered did not show a decrease in the intensity of disease greater than soaked treatment alone.

The decline in the intensity of the disease was great at soaking treatment for *P. fluorescens* P60 allegedly more effective to work on the root surface in the embedding medium compared to seedlings with drenching applications. This was consistent with the Kloepper *et al.*[17] and supported by the results of Soesanto research[8], that *P. fluorescens* has a strong ability to stick on the root surface.

Disease severity: Treatment with a solution of *P. fluorescens* P60 significantly affected the incidence of stem rot disease on dragon fruit seedlings caused by the fungus *S. rolfsii* (Table 1). It could be seen that the application could reduce disease incidence of 46.14%, followed by the drenching application that was 35.38%. However, if antagonistic P*. fluorescens* was applied by means of drenching and soaked, the incidence of disease suppression was not better than the application of soaked, both showed the same percentage of disease incidence.

This was presumably because the *P. fluorescens* effectively worked on the root zone. *P. fluorescencs* P60 able to defend themselves in rhizosper, able to increase the population, to produce compounds inhibiting pathogenic and capable to colonize plant roots[16,17,18].

Late population of sclerotium: Treatment of *P. fluorescens* P60 significant influenced the late population of sclerotia

Table 1: Effect of treaments on pathosystem component

Treatment	Incubation period (dai)	Disease intensity (%)[tn]	Disease severity (%)	Late population of sclerotium per polybag
Control	38.07[a]	20.00[b]	30.95[c]	90.67[d]
Soaked (10 min)	53.87[b]	6.67[a]	16.67[a]	22.67[a]
Drenching	51.80[b]	8.00[a]	20.00[b]	38.33[ab]
Soaked, drenching	54.20[b]	6.67[a]	16.67[a]	20.67[a]
Soaked in fungicide	47.23[b]	13.33[b]	30.00[c]	60.33[bc]

Figures followed by the same letter in the same column indicate no significantly difference on DMRT 5%. dai: Days after inoculation

Table 2: Effect of treatment on growth component

Treatments	Difference in seedling fresh weight (g)	Length of the longest root (cm)	Number of shoots	Shoot length (cm)
Control	104.97[a]	15.54[a]	2.10	28.02
Soaked (10 min)	160.47[b]	21.59[b]	2.83	38.95
Drenching	151.23[b]	20.35[b]	2.37	35.50
Soaked, drenching	153.70[b]	21.77[b]	2.33	30.35
Soaked in fungicide	141.47[b]	19.05[ab]	2.73	35.87

Figures followed by the same letter in the same column indicate no significantly difference on DMRT 5%

(Table 1). Treatment of *P. fluorescens* P60 on the concentration of 10^6 CFU mL^{-1} were able to decrease the number and intensity of the latesclerotia stem rot disease due to *S. rolfsii*, as 48-86 and 82-99%, respectively[8]. The average initial population sclerotia grown on PDA medium, namely 82 sclerotia/petri dish. *P. fluorescens* P60 giving treatment could lower latesclerotia population reached 74.79% with an average of 20.67 sclerotia/polybag namely in treatment soaked for 10 min and drenching on the planting medium.

Sclerotia suspected population decline due to the lengthy incubation period of the disease, as well as the intensity of the disease and the incidence of disease is low. The length of the incubation period, the intensity of the disease and the incidence of diseases affecting the number of sclerotia formed. This was supported by research of Soesanto *et al.*[14], which states that *P. fluorescens* P60 can inhibit the growth of pathogens, especially soil-borne pathogens and plant roots have the colonization ability.

Effect of treatments on growth component: Analysis of growth components showed significantly differences in between the fresh weight of the seedlings and the length of the longest root (Table2).

Difference in plant fresh weight: Treatment of *P. fluorescens* P60 gave real effect to the addition of plant fresh weight (Table 2). *P. fluorescens* P60 was able to increase the fresh weight of the seedlings up to 141.38% at soaked application for 10 min, followed by soaked 10 min and drenching, drenching, soaked in a solution of fungicide for 10 min and drenching and control with a percentage increase of fresh weight.

It happened because *P. fluorescens* P60 able to suppress stem rot caused by *S. rolfsii* so that seedlings could grow well and were associated with the ability to produce growth hormones that could stimulate plant growth. This was consistent with research of Santoso *et al.*[19], reported that *P. fluorescens* P60 is able to increase the fresh weight of onion crop amounted to 51.40%. Root colonizing bacteria (rhizobacteria) that exert beneficial effects on plant development via direct or indirect mechanisms have been defined as plant growth promoting rhizobacteria[20,21].

Length of the longest root: Treatment with *P. fluorescens* P60 significantly affected the root length of dragon fruit seedlings (Table 2). The longest root was found in the treatment of soaked for 10 min and drenching with an average of 21.77 cm, followed by treatment of the soaked for 10 min with an average length of roots 21, 59 cm and drenching with an average root length of 20.35 cm.

The high root length was allegedly because of their mechanism of *P. fluorescens* P60. In addition to the suppression of pathogens, it was also attributed to its ability to produce growth hormones that could stimulate the growth of plant roots and act as PGPR. In accordance with the said[21] that *P. fluorescens* acted as PGPR, so as to produce the growth hormone that could stimulate plant growth. It was also supported by the opinion[12], that *P. fluorescens* able to stimulate the growth of root system and inhibit harmful fungi and bacteria.

Number of shoots: Treatment of *P. fluorescens* P60 did not significantly affect the number of dragon fruit seedlings shoots. However, it could be seen in Table 2 that treatment of *P. fluorescens* P60 had greater average number of shoots when compared to control.

This was presumably because the seedlings treated with *P. fluorescens* P60 experienced disease so low that enhance the growth of seedlings and shoots can grow more. In addition, *P. fluorescens* is able to produce hormones that could stimulate plant growth. In accordance with the Nelson[21] said that *P. fluorescens* is PGPR, so as to produce the growth hormone that could stimulate plant growth.

The number of shoot in the treatment of soaked for 10 min with an average of 2.83, followed by treatment of soaked in a fungicide solution for 10 min and drenching, drenching and soaked for 10 min and drenching with an average number of shoots in succession, respectively, as 2.73, 2.37 and 2.33 shoots.

Table 3: Effect of treatments on phenolic compound content of dragon fruit seedlings

	Tissue analysis		
Treatments	Saponin	Tannin	Glycoside
Control	++	+	++
Soaked (10 min)	+++	++	+++
Drenching	+++	+++	+
Soaked, drenching	+	++	+++
Soaked in fungicide	++	+	+

Keterangan: +Low, ++High, +++Higher

Thus, it could be said that the treatment showed the best effect on the number of shoots on dragon fruit seedlings was soaked for 10 min. The application of *P. fluorescens* P60 antagonist was able to decrease the population levels of plant pathogens in the soil and promote plant growth test[6,8,14].

Shoot length: *P. fluorescens* P60 did not significantly effect on the average length of dragon fruit seedlings shoots. However, it could be seen in Table 3 that treatment of *P. fluorescens* P60 provided better effect of shoot length when compared to control. The longest shoot was indicated by soaked treatment for 10 min with a length of 38.95 shoots.

This was because *P. fluorescens* P60 was able to suppress the pathogen so that seedlings could grow and develop without the attack of pathogens and enhanced growth of seedlings. In accordance with the results[14,19,22] that the application of *P. fluorescens* P60 antagonist was able to reduce the level of populations of plant pathogens in the soil and promote plant growth.

In addition, an increase in the shoot long because *P. fluorescens* P60 was able to produce growth hormone compound as the "plant growth promoting rhizobacteria". It was evident that *P. fluorescens* P60 is able to produce auxin highest when compared with other similar isolates[7,14].

Phenolic compound content analysis: Testing qualitatively the content of phenolic compounds in the roots and stems of the dragon fruit seedlings showed a difference. *P. fluorescens* P60 showed their resilience system with an increase in phenol content (Table 3).

Results of tissue analysis dragon fruit seedling qualitatively indicated that the application of *P. fluorescens* P60 was able to increase the content of phenolic compounds (saponins, tannins and glycosides) in the seedlings tissue (Table 3). *P. fluorescens* P60 had ability to induce plant resistance.

The antagonistic bacteria *P. fluorescens* was able to induce plant resistance to pathogenic microbes[14]. This was also supported by Azizah[23] which states that the banana plant seeds are treated with extracts of *P. fluorescens* P60 and P32 show more resistant to fusarium wilt.

Secondary metabolites that were toxic and inhibit the growth of pathogens can induce plant resistance[14]. This mechanism does not inhibit the growth of plants and even to increase production and resistance to environmental stress on some crops[24].

The treatment could lengthen the incubation period as high as 29.76%. Inhibition of the incubation period was because the pathogen could not infect the seedlings. The seedlings could build a resistant character. The delay the incubation period because of the competition between pathogen and antagonist, so it took longer on pathogen to infect seedlings[11].

CONCLUSION

It is summarized that the use of *P. fluorescens* P60 effectively suppress stem rot caused by *S. rolfsii* on dragon fruit seedlings, by inhibiting the incubation period as 29.76%, lower the disease intensity as 66.65%, suppress the disease incidence of 46.14%, lower late sclerotia population of 74.79% and increase the content of phenolic compounds qualitatively. The application of *P. fluorescens* P60 is most appropriate to the nursery dragon fruit cuttings material by soaking in a solution of *P. fluoresc*ens P60 for 10 min. The use of *P. fluorescens* P60 can promote the growth of dragon fruit seedlings, covering the difference in seedlings fresh weight of 52.87%, root length as 40.09%, number of shoots as 34.76% and long shoots as 8.99%.

SIGNIFICANCE STATEMENT

This study discovers the possibility use of *Pseudomonas fluorescens* P60 that can be an alternative control for basal stem rot on dragon fruit seedlings. This study will help the researchers to uncover the crtical area of dragon fruit seedlings loss that many researchers were not able to handle. Thus a new theory on these liquid solution of antagonistic bacteria may be arrived at.

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