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Applications *Pseudomonas Fluorescens* Migula. to Control the Intensity of Purple Spot (*Alternaria Porri Ell. Cif.*) In Red Onion (*Allium Ascalonicum L.*)

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Abstract

Purple spot disease is caused by the fungus Alternaria porri Ell. Cif. which has the potential to cause damage and decrease shallot productivity by 3% -57%. Disease control in general is still emphasized by farmers regarding the use of chemical pesticides that can cause resistance to pests and diseases, and can cause pollution to the environment, even detrimental to human health. So it is necessary to have safe and friendly control for the environment, one of which is by using the biological agent Pseudomonas fluorescens Migula. The purpose of this study was to determine the effect of the concentration of P. fluorescens Migula. to suppress the intensity of purple spot disease caused by A. porri Ell. Cif. on shallots. This research was conducted from February to April 2023, at the UPTD Horticulture Seed Center located in West Java. This study used a randomized block design (RBD), consisting of 6 treatments and 4 replications. The treatment consisted of the application of P.

fluorescens Migula. with a concentration of 120 ml/l water, 140 ml/l water, 160 ml/l water, 180 ml/l water, Mankozeb 3 grams/l water and control. The results showed that the application of P. fluorescens Migula. influential in controlling the intensity of the disease with a concentration of 180 ml/l of water, the best in suppressing the attack of disease A . porry El. Cif. on shallots with an emphasis on disease intensity of 76.52%.

Keywords

Alternaria porri Ell. Cif. Pseudomonas fluorescens Migula. Allium ascalonicum L

Introduction

Shallot (*Allium ascalonicum* L.) is one of the most widely developed spice plants in Indonesia because it has many benefits for people's lives and has high economic value and is important for society. (Aryanta, 2019). Shallots are mostly used as a seasoning for cooking and have benefits as a traditional medicine because they have antiseptic and anti-microbial properties. The many benefits of shallot content motivate farmers to make various efforts to increase shallot crop production every year. (Nikirahayu, et.al., 2021).

Table 1.	The	productio	on and	produ	ctivity	data	for	shallots	in	Indonesia	in	2017-
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2021	are	shown	in	the	following	table:
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Year	Production (tons	Productivity (tons/ha)
2017	1,47	9.29
2018	1,50	9.58
2019	1,58	9.92
2020	1,81	9.71
2021	2	9.30

Source: Central Bureau of Statistics (2022)

Based on the shallot productivity data above, shows that the productivity of the shallot crop from 2017-2021 has fluctuated, influenced by many factors, one of which is purple spot disease caused by the fungus *A. porri* which has the potential to cause losses and decrease shallot productivity by 3% -57%. (Laksono, et. al., 2021). Disease control in general, farmers still emphasize the use of chemical pesticides that can cause disease and pest resistance and can cause environmental pollution, even detrimental to human health. (Warlinson Girsang, et.al., 2020). So it is necessary to have a relatively safe and friendly control for the environment by using the biological agent *P. fluorescens* Migula.

P. fluorescens Migula. is an antagonist bacteria that can induce systemic resistance and can increase the content of phenolic compounds which function to increase the resistance of plant tissues. (Prabowati, et al., 2021). *P. fluorescens* Migula. has also demonstrated its ability to control various plant pathogens, particularly soil-borne pathogens. Additionally, *P. fluorescens* Migula. acts as a PGPR bacterium which can increase the resistance of host plants and can stimulate plant growth.

Based on the research results of Laksono, et.al., (2021) proved that the

application of Migula fluorescens P concentration. 100 ml/l of water can suppress purple spot disease with an attacking intensity of 22.47% on shallot plants. Based on the results of research by Ulhaq, et al. (2019). Showed that the application of *P. fluorescens* Migula. 10 ml/plant can reduce the severity and incidence of downy mildew in corn plants by 27.77%. Based on the research results of Nasrun and Nurmansyah, (2016). showed *P. fluorescens* Migula. 100 g/l water can control bacterial wilt disease by 16.50–24.12% and can increase the growth and production of patchouli plants.

Based on this description, *P. fluorescens* Migula. can be used as a biological control microorganism and it is necessary to carry out research related to the control of purple spot disease on shallot plants at the UPTD Horticulture Seed Center. The purpose of this study was to determine the effect of the concentration of *P. fluorescens* Migula. against purple blotch disease and to determine the concentration of *P. fluorescens* Migula. the best in controlling purple spot disease (*A. porri* Ell. Cif.) on shallot plants.

Research Methods

This study used a randomized block design (RBD) method, in which there were 6 treatments and 4 replications, so there were 240 shallot plant populations. The 6 treatments that will be used to be tested are:

- 1. P1 = Control (No Treatment)
- 2. P2 = P. fluorescens Migula. 120 ml/l water
- 3. P3 = P. fluorescens Migula. 140 ml/l water
- 4. P4 = *P. fluorescens* Migula. 160 ml/l water
- 5. P5 = *P. fluorescens* Migula. 180 ml/l water

6. P6 = Mancozeb 3 gram/l water (Positive Control or Comparison) According to Freund and Wilson (1996) in Vina, RF, et al., (2014) Randomized block design The linear model is as follows:

 $Yij = \mu + ti + \beta j + \Sigma ij$

Information

1. Yij : Observation value (response) from the i-the treatment and j-th repetition

- 2. μ : General average value
- 3. ti : The effect of the i-th treatment (i=1, 2, 3, t....)
- 4. βj : Effect of the jth treatment

5. Σij : The effect of random factors on the first treatment in the J group If the F test shows a significant difference, then to differentiate the averages of each treatment, it is necessary to carry out a follow-up test using Duncan's multiple range test method at the 5% significance level with the following formula : LSR = SSR x S \overline{x} Where LSR = Last Significant Rangers SSR = Studentized Significant Rangers S \overline{x} = Mean Standard Error = $\frac{\sqrt{KT Galat}}{r}$

Preliminary Test

1. Observation and Identification of Altenaria porri Ell. Cif

Observation of shallot plants was carried out on land belonging to the UPTD Horticulture Seed Center located in West Java. Identification of the disease was carried out at the Faperta Biology Laboratory, Islamic Nusantara University, Bandung, West Java, using a microscope as a tool for this identification.

2. Preparation of PSA Media (Potato Sucrose Agar)

Making PSA media is done by boiling potatoes until soft which has been cleaned and diced as much as 600 g, then separate the potatoes from the cooking water, then add 48 g of agar-agar and 20 g of sucrose sugar, and add mineral water until the total reaches 1 L., then cook until boiling and homogeneous. After that, remove and pour into a petri dish, test tube, and Erlenmayer, then wrap with aluminum foil to be sterilized by placing it in the autoclave at 121° C for \pm 2 hours/until the autoclave beeps. PSA media that has been sterilized is stored at room temperature \pm 24 hours before use.

3. Isolation and Identification of Causes of Disease

Isolation of the pathogen was carried out by cutting the infected leaf with a size of about 1 cm, then growing it on a PSA medium in vitro, aiming to get a pure culture. The cut leaf samples were soaked in 70% alcohol solution for \pm 3 minutes, again soaked in 1% NaoCL solution \pm 3 minutes further soaked in 70% alcohol \pm 1 minute, and finally rinsed using sterile water for 3 seconds and repeated as many times. 3 times. Dry on sterile filter paper in the Petri dish, after drying the leek samples are put into the Petri dish-containing PSA media and incubated at room temperature for 7 days or until the colonies fill the Petri. After the growing hyphae are purified and multiplied on PSA media which will be used as material for microscopic identification activities, then multiply by rejuvenation for sample preparation in carrying out the antagonist test.

Research Implementation in the Field

1). Polybag preparation, using polybags of 240 polybags measuring 30 cm \times 30 cm. 2). The planting medium used is soil, husks and manure with a ratio of 1:1:2 then put into a polybag. 3). The preparation of shallot seeds to be used is shallot seeds that have been stored for 2-4 months because their germination has

reached 80%. The seeds used were the Brebes Bima variety, with 240 tubers. Before planting the top of the tuber is cut to accelerate the growth of shoots 4). Planting is done by first mixing the husks, soil, and manure and then putting them in a polybag. Each polybag is given a hole then one onion seed bulb is planted in each polybag. The size of the polybag used is 30×30 cm with a spacing of $20 \times$ 20 cm with the required land area of 45 m. 5). Onion plant maintenance consists of supplementary fertilization, weeding, and watering. Watering is done 2 times a day ie morning and evening until the plants are 10 days old. Shallot fertilization was carried out 3 times, namely the first fertilization was carried out when the plants were 2 MST using NPK fertilizer 10 g/l water per plot, and the second followup fertilization was carried out when the plants were 4 MST using NPK fertilizer 10 g/l water per plot, the third fertilization on age 6 MST. Weeding is done according to the conditions of weed growth in the field. 6). Application of the Biological Agent *P. fluorescens* Migula. carried out after the intensity of disease attacks by spraying using a hands prayer for 3 applications, spraying is done once a week. 7). Harvesting was carried out at 70 DAP with the characteristics of 60% of the neck being soft, the plants drooping, and the leaves yellowing. Harvesting is by pulling the shallot bulbs down to the roots, then the roots and the remaining soil that is lifted are cleaned and the leaves are cut off.

Observation Parameters

Observations were made on 5 samples of shallot seedlings from each plot, sampling was taken using a zig-zag system. The parameters observed are:

1. Attack Intensity

Observation of attack intensity was carried out twice, the first was observation before the application of *P. fluorescens* Migula. conducted to determine the intensity of the initial attack of *A. porri* and the second observation of the intensity of the attack after the application of *P. fluorescens* Migula. conducted to determine the intensity level of *A. porri* attacks.

After being observed and given a score, the intensity is calculated based on the damage scale. According to (Asri, et.al., 2022). Observation of the intensity of disease attacks is carried out using the formula:

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

Information

- I: Disease intensity
- n: The number of infected leaves in each attack category
- V: Scale value in each category
- N: The number of leaves observed
- Z: The highest scale value

2. Leaf Height

Leaf height was measured from the ground surface to the first leaf margin. Observations of height were measured using a ruler with an observation time of 5-8 MST.

3. Number of Leaves

Observation of the number of leaves was carried out from 5 WAP to 8 MST. The number of leaves observed was all the leaves in each sample.

4. Total Weight of Tuber/plot

Observation of yields was carried out to determine the effect of the application of *P. fluorescens* Migula. on the results of the weight of the shallot bulbs by weighing all the bulb samples in the treatment plots which were harvested when the shallots were 70 HST, using a digital scale then weighing and recording the results.

Results And Discussion

Laboratory Test Results

1. Identification of Altenaria porri El. Cif. Causes of Disease

Identification of the disease was carried out to find out the pathogens present on the leaves of the plant. A sampling of the UTPD Horticulture Seed Center was carried out by taking leaf samples that had symptoms of spots on the leaves. The samples taken are shown in the image below:



Figure 1. Samples of Leaves with Purple Spots Source: 2023 Personal Documentation

The identification test used positive leaves with symptoms of purple spot disease, then identification was carried out at the Biology Laboratory of the Faculty of Animal Husbandry at the Islamic Archipelago University using a microscope as a tool for this identification. Following are the results of the identification of *Altermaria porri* Ell. Cif. On shallot plant leaves that are positive for pathogens:



a. Colony b. Conidia Figure 2. a colony and b. conidia Source: 2023 Personal Documentation

2. Attack intensity

Observations began one week after application as much as 4 observations before application and after application, observations before application were carried out at 5 WAP, and application after application started from 6 MST to 8 MST. The results of observations on the average intensity of *Altenaria porri* Ell disease. Cif is presented in the following table:

Cif)					
Treatment	5 MST	6 MST	7 MST	8 MST	
P1	2,14ª	7,97ª	16,94ª	23,51ª	
P2	0,69 ^b	5,81 ^{ab}	5,45 ^b	12,03 ^b	
Р3	1,32 ^{ab}	5,28 ^{ab}	6,06 ^b	11,68 ^{bc}	
P4	0,79 ^b	5,57 ^{ab}	6,24 ^b	10,28 ^{bc}	
Р5	1,28 ^{ab}	3,75⁵	4,14 ^b	5,52 ^d	
P6	0,72 ^b	5,29 ^{ab}	5,29 ^b	9,26 ^{bc}	

Table 4. Results of Analysis of Average Intensity of Disease (Altenaria porri Ell.

Information

Numbers followed by the same letters in the same table column were not significantly different according to Duncan's multiple range test at the 5% level.

Treatment

- P1 = Control (No Treatment)
- P2 = *P. fluorescens* Migula. 120ml/l water
- P3 = *P. fluorescens* Migula. 140ml/l water
- P4 = *P. fluorescens* Migula. 160ml/l water
- P5 = P. fluorescens Migula. 180ml/l water
- P6 = Mancozeb 3 gram/l water (positive control or comparison)

Based on Duncan's multiple range test at a 5% significance level, the results of the analysis of the average intensity of Altenaria porri Ell disease. Cif. In the first observation 5 MST before application, all treatments were significantly different from P1 (control), except for treatments P2, P4, and P6 at 5 MST observations. This is because there is no treatment application to prevent *Altenaria porri* Ell disease. Cif.

Observations at 6 MST showed that all treatments were significantly different from P1 (Control) except for treatment P5 (*P. fluorescens* Migula. 180 ml/l

water). This is due to P. fluorescens Migula.

According to (Gratitude, et.al., 2022). *P. fluorescens* Migula. has the ability to suppress several pathogens, one of which is soil-borne pathogens, and can increase the content of phenolic compounds which function to increase the resistance of plant tissues. *P. fluorescens* Migula. can suppress the growth of fungi and bacteria in a way mechanism of antibiosis, parasitism, and competition.

P. fluorescens Migula. able to suppress plant diseases by producing several secondary metabolites including antibiotics, siderophores, and hydrogen cyanide. These bacteria possess a combination of effective biological control mechanisms and unique abilities to enter the plant vascular system, reach various parts of the plant system and act as systemic biocontrol agents against various fungal and bacterial diseases. (Prabowati, et al., 2021).

Observations at 7 MST showed that all treatments were significantly different from P1 (control), all treatments were *P. fluorescens* Migula. showed no significant difference with the Mankozeb 3 g/l water treatment (positive control or comparison). This proves that P and mancozeb fungicides have the same effect on reducing the intensity of *Altenaria porri* Ell disease. Cif. The fungicide with the active ingredient mancozeb has a working mechanism, namely by inhibiting the activity of enzymes in the fungus by producing an enzyme layer containing metal elements which act as the formation of an energy source for plant metabolic activities. Mancozeb fungicide has a direct effect on fungal biochemical processes which can inhibit the spore development process in plants (Hajijah, et.al., 2022). Observations at 8 MST showed that all treatments were significantly different from P1 (control). All treatments of *P. fluorescens* Migula. Each of them was not significantly different from treatment P6 (mancozeb 3 grams/liter of water) or comparison, except for treatment P5 (*P. fluorescens* Migula. 180 ml/l water). which showed the lowest disease intensity of 5.52% which was able to suppress disease intensity by 76.52%.

Effect of *P.fluorescens* Migula. not immediately visible in a short time, but takes time to provide environmental stability in suppressing the development of pathogens. *P. fluorescens* is a root colonizing bacterium that produces salicylic acid and phytoalexins which induce plant resistance to pathogens. Additionally, *P. fluorescens* Migula. has the ability to induce systemic resistance which has demonstrated its ability to control several plant pathogens, particularly soil-borne pathogens. *P. fluorescens* Migula. has the properties of "Plant Growth Promoting Rhizobacteria" (PGPR), and produces antibiotics and siderophores. Additionally, *P. fluorescens* Migula. being able to colonize plant roots can increase the content of plant phenolic compounds. This bacterium has a mechanism of parasitism, competition, and antibiosis so that it can suppress the intensity and spread of disease in plants. (Lestari, et.al., 2021).

3. Leaf Height

Observation of plant height began when the plants were 5 WAP to 8 MST after the application of treatment. Based on the results of observations and analysis

of variance on the average height of shallot plants, it was shown that the various treatments of *P. fluorescens* Migula were given. significant effect on shallot (*Allium ascalonicum* L.) plant height. The results of the observation analysis test are presented in the table below:

Table 5. Results of Analysis of Average Leaf Height in Shallot Plants (Altenaria

Treatment	5 MST	6 MST	7 MST	8 MST
P1	33,64ª	34,79ª	32,37 ^b	32,63 ^b
P2	34,43ª	35,44ª	37,25ª	37,43ª
Р3	34,03ª	35,77ª	37,88ª	38,42ª
P4	34,93ª	34,83ª	38,68ª	38,66ª
P5	34,66ª	33,97ª	37,81ª	39,76ª
P6	33,78ª	34,86ª	39,19ª	40,49ª

porri Ell. Cif)

Information

Numbers followed by the same letter in the same column were not significantly different according to Duncan's multiple range test at the 5% level.

Treatment

- P1 = Control (without *P. fluorescens* Migula treatment.)
- P2 = P. fluorescens Migula. 120 ml/l water
- P3 = *P. fluorescens* Migula. 140 ml/l water
- P4 = *P. fluorescens* Migula. 160 ml/l water
- P5 = *P. fluorescens* Migula. 180 ml/l water
- P6 = Mancozeb 3 gram/l water

Based on Duncan's multiple range test at a 5% significance level, the results of the analysis of the average height of shallot (*Allium ascalonicum* L.) plants in the observation of 5 MST and 6 MST before application and after application, all treatments showed no significant difference with treatment P1 (control). This was due to the absence of treatment applications and was suspected from the observation of 6 MST *P. fluorescens* Migula. does not affect the height of shallots.

Observations at 7 WAP and 8 MST showed that all treatments were significantly different from P1 (control), but all treatments were *P. fluorescens* Migula. each was not significantly different from treatment P6 (mancozeb 3 grams/liter of water) or the comparison. This proves that *P. fluorescens* Migula. and fungicides have the same effect on the growth of shallot plants.

According to (Tabriji, et.al., 2016). *P. fluorescens* Migula. (PGPR) which can increase growth and yields, and can produce several plant growth hormones, namely Indole Acetic Acid (IAA). The IAA hormone plays a role in cell enlargement, and formation of phloem and xylem tissues, which can affect root elongation and nitrogen fixation.

4. Number of Leaves

Observation of the number of leaves began when the plants were 5 WAP to 8 MST after the treatment application. Based on the analysis of variance and the results of observations on the average number of leaves of the shallot plant, it was shown that the various treatments of *P. fluorescens* Migula were given. effect on onion plant height. The results of the observation analysis test are presented in the table below:

Table 6. Results of Analysis of the Average Number of Leaves on Shallot Plants (Altenaria porri Fll, Cif).

Treatment	5 MST	6 MST	7 MST	8 MST
P1	40,95ª	31,55ª	24,00 ^b	18,00 ^b
P2	40,00ª	35,95ª	36,35ª	36,35ª
Р3	39,00ª	32,00ª	33,80ª	28,20ª
P4	43,83ª	29,35ª	37,85ª	30,95ª
P5	41,93ª	32,70ª	43,70ª	35,90ª
P6	39,00ª	28,65ª	34,05ª	28,35ª

Information

Numbers followed by the same letter in the same column were not significantly different according to Duncan's multiple range test at the 5% level.

Treatment

P1 = Control (No Treatment)

P2 = *P. fluorescens* Migula. Migula. 120 ml/l water

P3 = P. fluorescens Migula. 140 ml/l water

P4 = *P. fluorescens* Migula. 160 ml/l water

P5 = *P. fluorescens* Migula. 180 ml/l water

P6 = Mancozeb 3 g/l water (Positive Control or Comparison)

Based on Duncan's multiple range test at a 5% significance level, the results of the analysis are the average number of shallots. At 5 MST and 6 MST observations before application and after application, all treatments showed no significant difference with the P1 treatment (control). This was due to the absence of treatment applications and was suspected from the observation of 6 MST *P. fluorescens* Migula. does not affect the number of shallots.

Observations at 7 WAP and 8 MST showed that all treatments were significantly different from P1 (control), but all treatments were *P. fluorescens* Migula. each was not significantly different from treatment P6 (mancozeb 3 grams/liter of water) or the comparison. This proves that *P. fluorescens* Migula. and mancozeb fungicide had the same effect on shallot plant growth.

According to Lala and Max Tulung, (2019). *P. fluorescens* Migula. has the property (PGPR), which can colonize plant roots and can increase the content of plant phenolic compounds, and can produce antibiotic compounds and siderophores. This bacterium has a mechanism of parasitism, competition, and antibiosis so that it can suppress the intensity and spread of disease in plants.

5. Total tuber weight/plot

Observation of shallot bulb weight yield was carried out on plants aged 70 days after planting. Based on the results of Duncan's multiple range test analysis at a 5% significance level by the application of *P. fluorescens* Migula. showed an effect on the weight yield of shallot bulbs. Following are the results of observing the average weight of shallot bulbs presented in Table. the following 6:

Table 7. Results of Analysis of the Average Number of Bulbs in Shallot Plants (*Altenaria porri* Ell. Cif).

Treatment	Average Weight tubers Onion Red (gram)/ plant
P1 (Control No treatment)	96,78 ^b
P2 (<i>P.fluorescens</i> Migula. 120 ml/l water)	302,13ª
P3 (<i>P.fluorescens</i> Migula. 140 ml/l water)	328,85ª
P4 (<i>P.fluorescens</i> Migula. 160 ml/l water)	339,15ª
P5 (<i>P.fluorescens</i> Migula. 180 ml/l water)	350,65ª
P6 (Mankozeb 3 gram/l water)	394,95ª

Note: Numbers followed by the same letter in the same column are not significantly different according to Duncan's multiple range test at the 5% level.

Based on the results of Duncan's multiple range test analysis at the 5% level, the average weight of shallot bulbs showed that all treatments were significantly different from treatment P1 (control). The results of the highest tuber harvest average were shown in treatment P6 (Mankozeb 3 grams/liter of water) or comparison, but not different from treatment P5 (*P. fluorescens* Migula. 180 ml/l water). This proves that *P.* fluorescens Migula. and mancozeb fungicide both affected the yield of tuber weight in shallot plants.

According to (Ningsih and Nurul, 2021). That *P.fluorescens* Migula. (PGPR) which can directly increase plant growth through the growth hormone Gibberellins (Gac) and Indole 3-Acetic Acid (IAA). Besides that, it can increase yields for shallot plants. This is because (PGPR) plays a role in accelerating the absorption of nutrients through plant roots so that by applying PGPR bacteria it can meet the needs of macro and micronutrients and stimulate growth in the vegetative phase.

Conclusions and Recommendations

1. Based on the results of the research that has been done, the following conclusions can be obtained:

2. Application of Pseudomonas fluorescens Migula. effect in controlling purple spot disease caused by Alternaria porri Ell. Cif. on onion plants.

3. Pseudomonas fluorescens Migula. with a concentration of 180 ml/l water had the best effect on controlling purple spot disease on shallot plants with an emphasis on disease intensity of 76.52%.

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