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The Effectiveness of *Trichoderma viride* in suppressing stem rot (*Sclerotium rolfsii*) and enhancing growth and yield of Porang

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Abstract

Porang is a commodity that commands a high price due to the numerous benefits of its tubers. Root rot caused by *Sclerotium Rolfsii* is one of the main diseases affecting Porang. One control measure that can be implemented is the use of the biological agent *Trichoderma Viride*. The aim of this study is to determine the most effective dose of *T. viride* in a solid formulation for controlling stem rot caused by *S. rolfsii*. This research was conducted at the Forecasting for Plant Pest Organisms facility in Jatisari, Karawang, from September 2021 to April 2022. The research design employed was a Completely Randomized Block Design (CRBD) with a single factor. The doses used were 0 (as control), 50, 75, 100, 125, and 150 g per plant. *T. viride* was propagated using rice as the medium. The study involved six doses of *T. viride in a solid formulation* with four replications. Data analysis was performed using ANOVA at a 5% significance level, followed by DMRT at the same level. *T. viride* at a dose of 150 g per plant resulted in the best suppression of root neck rot intensity at 69.17% and suppression of tuber rot percentage at 69.46%. The application of *T. viride* was able to increase the growth and yield of Porang.

Keywords: Porang, *Sclerotium Rolfsii*, Stem rot, *Trichoderma viride*

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1. Introduction

Porang (*Amorphophallus muelleri* Blume) is a type of plant from the Araceae family (taro). According to Saleh et al. (2015) Porang tubers contain carbohydrates, proteins, fats, vitamins, and fiber. In addition, Porang tubers contain glucomannan, which can be used as a functional food ingredient beneficial for health. Porang tubers are utilized in various applications, including food products, syrup thickeners, tablet fillers, coating materials, adhesives for glue and wall paint, waterproof coatings, woven reinforcements, microbial growth media, and papermaking materials. The many benefits of Porang tubers make them a very promising export commodity. However, there are several obstacles to Porang cultivation, one of which is plant disease.

Porang stem rot (BPBP), caused by *Sclerotium rolfsii*, is the main disease affecting Porang. Based on Shao et al. (2015), symptoms of the disease include leaf color changes from light green to yellow, discoloration at the stem base, wilting, bulb rotting, and ultimately plant death, as observed in Konjac affected by Phytophthora nicotianae in China. Infection by *S. rolfsii* leads to tuber rot, which reduces production in terms of both quality and quantity. According to Aktar et al. (2009), continuous control of BPBP using synthetic pesticides can pollute the environment, harm non-target organisms, and leave residues in agricultural products that negatively impact human health. The biological agent *Trichoderma viride* has been proven effective in suppressing the development of several diseases through its antagonistic mechanisms. The results of Singh et al. (2013) showed that soaking suweg (*Amorphophallus paeoniifolius*) seedlings in a *T. viride* solution can increase resistance to leaf blight.

Additionally, research by Mahabbah et al. (2014)) demonstrated that the application of *Trichoderma* spp. is effective in suppressing the growth of *S. rolfsii*. Furthermore, *Trichoderma* spp. has been shown to stimulate vegetative plant growth, as evidenced by research from Suanda (2016), and to increase tuber weight, as shown in studies by Barus et al. (2018). However, there has not been extensive research on controlling stem rot disease in Porang. This study aims to examine the effectiveness of *T. viride* in suppressing BPBP and in increasing the growth and yield of Porang.

2. Material and Methods

The in vitro antagonism test was conducted from September 30 to October 14, 2021, at the Phytopathology Laboratory, Center for Plant Pest Forecasting (BBPOPT), Jatisari, Karawang, West Java. The methods used were the dual culture test and the steam culture test. The dual culture variable observed was the diameter of the pathogen colonies, approaching the mean diameter. The percentage of inhibition was measured after all pathogens in the control treatment filled the Petri dish and was calculated using Equation 1 (BSN, 2014).

Obstacle (%) = $\frac{r1-r2}{r1}\ x\ 100\%$ .... [1]

Where r1: radius of the pathogen in the control treatment r2: radius of the pathogen in the treatment growing toward the biological agent

The steam culture test variable observations of the inhibition of pathogen fungal growth using the steam sterilization method were conducted by measuring the diameter of the colonies until the day the pathogen in the control treatment filled the Petri dish. The level of inhibition was calculated by comparing the colony diameter in the biological agent treatment with that in the control. That was calculated using Equation 2 (BSN, 2014).

$$\text{Obstacle (\%)} = \frac{d1-d2}{d1}\ x\ 100\% \quad \text{[2]}$$

Where d1: diameter of the pathogen in the control treatment d2: diameter of the pathogen in the treatment

The field efficacy test was conducted in the Greenhouse of BBPOPT from October to April 2022. This study utilized a Completely Randomized Block Design (CRBD) with a single factor. The factor examined was the dosage of *T. viride* in a solid formulation, consisting of 0 (control), 50, 75, 100, 125, and 150 g per plant. Each treatment included four replications. The efficacy test began with the propagation of *T. viride* on rice media. Rice was chosen as the medium because it provides a good carbon source for the growth of *T. viride*, allowing it to thrive optimally before application. The application of *T. viride* was conducted one week before planting, as it was expected that *T. viride* would adapt to its environment and reproduce prior to planting.

Porang planting was carried out in the greenhouse on October 19, 2021, at an altitude of 50.8 meters above sea level, with an average temperature of 36°C. Planting occurred when the tubers finished their dormancy and during a period of high rainfall. According to the research by Siswanto & Karamina (2016), Porang prefers high rainfall, between 300-500 mm per month, particularly during the vegetative growth stage. The growing medium used was a mixture of soil and compost at a ratio of 2:1. Inoculation of the pathogen *S. rolfsii* was performed in the growing medium, as *S. rolfsii* is a soil-borne pathogen whose initial inoculum resides in the soil. Observations were made on 240 sample plants across eight greenhouses, each containing six treatments arranged randomly. Each replication consisted of 10 plants. Harvesting was conducted on March 29, 2022, when the plants were 23 months old. The observed variables in this field efficacy test were as follows.

The incubation period of *S. rolfsii* is the time required from the initial inoculation of *S. rolfsii* until the first symptoms of stem rot appear. The intensity of the disease was observed weekly, starting from 2 months after planting (MST) until harvest (23 MST). Observations were made visually by assessing the symptoms of stem rot and determining the severity scale. The intensity of the disease was calculated using Equation 3 (Kementan, 2018).

$$I = \frac{\sum_{1=0}^{z}(ni \times vi)}{Z \times N} \times 100 \quad \text{[3]}$$

Where I : Intensity of Attack (%); ni : number of plants or plant parts exhibiting damage at severity level vi; vi : severity level value of the iii-th sample; N : total number of plants or plant parts observed; Z : highest severity level value;

Severity Scale for the Disease:0 : no symptoms; 1 : area of symptoms at the base of the stem >1% and <5%; 3 : area of symptoms at the base of the stem >5% and <25%; 5 : area of symptoms at the base of the stem >25% and <50%; 7 : area of symptoms at the base of the stem >50% and <75%; 9 : area of symptoms at the base of the stem >75% and 100%; The incidence of the disease was observed weekly from 2 MST to 23 MST. Observations were conducted visually by checking for the presence of collar rot symptoms. The incidence was calculated using Equation 4 (Kementan, 2018).

$$IP = \frac{n}{N}\ x\ 100\% \quad \text{[4]}$$

Where IP = disease incidence; n = number of diseased plants; N = total number of observed plants

The percentage of tuber damage was observed after the tubers were weighed. Observations were conducted visually by cutting the tubers in half and assessing the percentage of rot. To confirm the cause of tuber rot, isolation procedures were performed. There were also measurements of plant height, weight of Porang tuber, and weight of Porang bulbils.

3. Results and Discussion

3.1. Results of in Vitro Antagonism Testing

The inhibitory effect in dual culture tests was observed by measuring the radius of the pathogen approaching the diameter of the Petri dish. In contrast, the inhibitory effect in vapor culture tests was assessed by measuring the diameter of the pathogen. The comparison was made with the pathogen that was not treated with *T. viride* (control). The calculation of the inhibitory effect was concluded when the control pathogen fully covered the Petri dish. The percentage of inhibition by *T. viride* is presented in Table 1. The highest inhibition of *T. viride* in both the dual culture test and the vapor culture test occurred at four Days After Inoculation (DAI), with values of 69.44% and 37.70%, respectively.

The antagonistic mechanism is the method used by antagonistic microbes to suppress the growth of pathogenic microbes. The antagonistic mechanism can be observed both macroscopically and microscopically. The results of the dual culture test between *T. viride* and *S. rolfsii* can be seen in Figure 1.

In the dual culture method, it can be observed that the growth of *T. viride* colonies is faster than that of *S. rolfsii*. This results in the inhibition of *S. rolfsii* colony growth. The faster colony growth indicates competition for nutrients and living space between *T. viride* and *S. rolfsii*. This is consistent with the study by Oszust et al. (2020), which showed that Trichoderma is capable of colonizing various substrates and suppressing slower-growing pathogens. After the interaction of the two fungi, microscopic observations were conducted to examine the antagonistic mechanism. Observations were made at 40x magnification. The results can be seen in Figure 2.

Table 1. Percentage of inhibition by *T. viride* in dual culture and vapor culture tests

Days After Inoculation	Percentage of inhibition (%)	
	Dual culture test	vapor culture tests
1	40.00	0.00
2	28.00	9.52
3	59.26	31.43
4	69.44	37.70
5	69.44	35.14
6	69.44	34.17

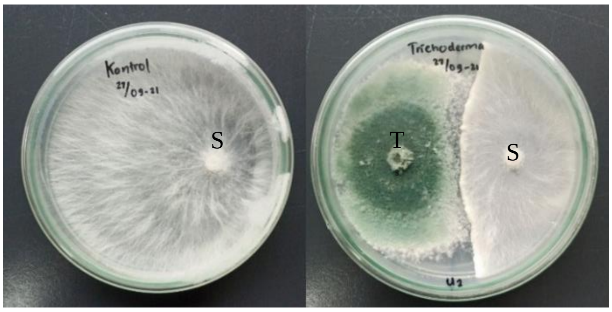


Figure 1. Results of the antagonistic test using the dual culture method at 4 HSI. (S) *S. rolfsii*, (T) *T. viride*

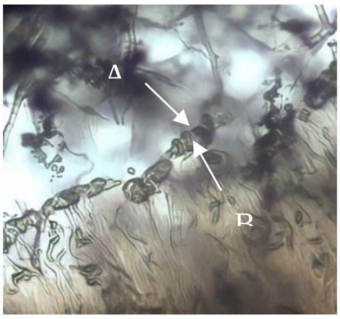
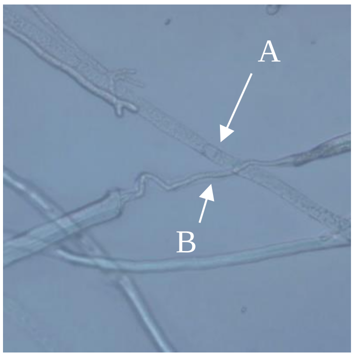


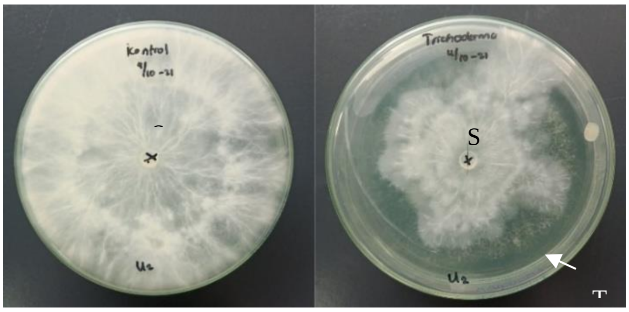
Figure 2. Microscopic appearance of the interaction between *T. viride* hyphae coiling around *S. rolfsii* hyphae. (A) *T. viride* hyphae (B) Hifa *S. rolfsii* hyphae.

In Figure 2, it can be seen that *T. viride* hyphae are coiling around *S. rolfsii* hyphae. This interaction indicates the presence of parasitic activity. According to Mukherjee et al. (2012), Trichoderma is a necrotrophic parasite that employs mechanisms for prey sensing, chemotaxis, adhesion to hosts, and physical attack through intense branching and coiling around host hyphae. Additionally, (Moreno-Ruiz et al., 2020) state that Trichoderma can form penetration structures known as appressoria. Within the appressoria, there is glycerol that can induce penetration into host cells. After *T. viride* hyphae parasitize *S. rolfsii*, degradation of the pathogen's cell wall occurs due to the activity of hydrolytic enzymes and antifungal compounds produced by *T. viride*, leading to pathogen death. This is consistent with the research by Paulitz and Bélanger (2001), which indicates that *T. viride* can produce chitinase, damaging the chitin cell wall of *S. rolfsii*. In Figure 3, *S. rolfsii* hyphae are shown to shrink due to the attachment of *T. viride* hyphae. This shrinkage causes the pathogenic cells to lose their permeability properties, preventing them from selectively regulating substances entering and exiting the cells. As a result, secondary metabolites can enter the cells, leading to lysis followed by cell death. This aligns with the findings of Muhibuddin et al. (2021), which state that *T. viride* hyphae can coil around *S. rolfsii* hyphae, causing morphological deformities and disorganization of the cell wall structure.

The steam test aims to determine the presence of volatile compounds produced by biological agents to suppress pathogen growth. Volatile compounds are low molecular weight substances (100-500 Dalton), easily evaporated, and typically emit a distinct aroma. In Figure 4, it can be observed that the treatment of inverting a petri dish containing *S. rolfsii* culture over *T. viride* affects the colony growth diameter of *S. rolfsii* compared to the control, which was inverted over PDA medium (without biological agents). This indicates the presence of an antibiosis mechanism or inhibition of pathogen growth.



Description: (A) Hyphae of *T. viride* (B) Hyphae of *S. rolfsii*
Figure 3. Microscopic appearance of *S. rolfsii* hyphae showing morphological deformation due to the wrapping of *T. viride* hyphae



Description: (S) *S. rolfsii*, (T) *T. viride*
Figure 4. Results of the antagonism test using the steam method on 6 HSI

In Figure 4, morphological changes in the hyphae of *S. rolfsii* are evident. In the treatment with *T. viride*, the growth of *S. rolfsii* hyphae becomes thinner compared to the control. These morphological changes in the hyphae are caused by the activity of volatile compounds produced by *T. viride*. The inhibited growth of *S. rolfsii* in the steam test is due to the presence of volatile compounds generated by *T. viride*. According to the research by Nemčovič et al. (2008), *T. viride* produces volatile compounds such as 3-octanone, 3-octanol, *beta-sesquiphellandrene*, 2-*pentylfuran*, and *1-octen-3-ol*. Wheatley et al. (1997) reported the presence of *2-heptanone* in *T. Viride*.

3.2. Effectiveness of BPBP Suppression by *T. Viride*

Observations indicate that the early symptoms of BPBP are brown spots at the base of the stem, marked by the presence of white mycelium or sclerotia around the stem's base. The subsequent symptom is the rotting of the stem base, accompanied by an increasing number of sclerotia. The rot at the base of the stem becomes more widespread, ultimately leading to plant death. The final symptoms can be found on the rotting tubers. The BPBP symptoms can be seen in Figure 5.

The results of the ANOVA on the incubation period of *S. rolfsii*, the intensity and incidence of BPBP, as well as the percentage of tuber rot, are presented in Table 2. The results of the analysis of variance indicate that the dose of *T. viride* does not have a significant effect on the incubation period of *S. rolfsii*. This may be due to the less than optimal adaptability and development of T. viride, resulting in insufficient suppression of *S. rolfsii* growth. According to Amaria et al. (2018), good adaptability and development of Trichoderma spp. can inhibit pathogen infection during penetration, colonization, and degradation stages, thereby affecting the duration of the pathogen's incubation period.

Figure 5. BPBP Symptoms: (A) brown spots and presence of sclerotia, (B) rotting stem base, (C) plant death, (D) rot on tubers

Table 2. Response of incubation period, intensity, incidence, and tuber rot to different doses of T. viride.

Dose (g per plant)	Incubation Period of *S. rolfsii* (MST)	Intensity of BPBP (%)	Incidence (%)	Tuber Rot (%)
0	17.83±0.80	2.66±1.56 b	12.39±5.25 b	9.43±7.68 b
50	17.44±2.26	1.35±0.85 a	5.11±2.18 a	4.23±2.08 a
75	19.88±0.25	0.91±0.58 a	4.32±1.41 a	3.18±4.28 a
100	18.13±1.31	1.09±0.44 a	4.09±1.86 a	5.88±2.32 a
125	19.03±0.68	1.01±0.65 a	5.45±4.38 a	5.10±4.33 a
150	18.44±1.26	0.82±0.74 a	5.11±2.76 a	2.88±3.32 a
Sig. (*p*)	0.085	0.032	0.019	0.003
KK (%)	6.14	57.95	41.27	76.13

Notes: Numbers followed by the same letter in the same column indicate no significant difference in the DMRT (Duncan's Multiple Range Test) at the 5% level.

Based on the analysis of variance, it can be concluded that the treatment with *T. viride* doses increases the suppression of the intensity of tuber rot in Porang. Porang without *T. viride* treatment showed the highest average intensity of BPBP at 2.66%, while the lowest intensity resulted from the treatment of *T. viride* at a dose of 150 g per plant, which was 0.82%. The highest dose of *T. viride* was also able to provide the greatest suppression. It is suspected that as the dose increases, the number of *T. viride* propagules also increases, leading to higher production of toxins and enzymes. The suppression of the intensity of tuber rot may be due to the success of *T. viride* in inhibiting the growth of the pathogen *S. rolfsii*. According to Rodriguez-Kabana et al. (1978), *T. viride* can produce proteolytic enzymes. The activity of these enzymes is involved in the biological control of *S. rolfsii*. According to Goldman et al. (1994), protease enzymes can degrade cell walls and protein membranes, followed by the lysis of pathogens, allowing *T. viride* to obtain nutrients. Furthermore, based on research by Muhibuddin et al. (2021), *T. viride* produces steroids and alkaloids that can suppress the growth of pathogenic fungi.

Based on the analysis of variance, it can be concluded that different doses of *T. viride* have a significant effect on the incidence of BPBP. The highest average incidence of BPBP was found in the control group, at 12.39%. The lowest incidence, at 4.09%, was observed with the treatment of 100 g per plant. The low incidence of the disease is related to T. viride's ability to induce plant resistance. In addition to producing secondary metabolites, *T. viride* can induce plant resistance by fulfilling the nutritional needs of the plants from the soil. When nutritional needs are met, plants become more resilient to pathogen attacks. According to Barus et al. (2018), *T. viride* can enhance the soil ecology around the plant roots by suppressing pathogenic soil fungi and acting as a decomposer to make nutrients available for the plants.

The analysis of variance results show that the application of *T. viride* can reduce the percentage of Porang tuber rot compared to the control. The highest percentage of tuber rot was observed in the control group, at 9.43%, while the lowest percentage of tuber rot was found with the treatment of *T. viride* at a dose of 150 g per plant, yielding 2.88%. The percentage of tuber rot is proportional to the intensity of stem base rot. This is because a higher intensity leads to a wider attack by *S. rolfsii*, allowing the infection to spread to the tubers and cause rot. The successful suppression of stem base rot intensity by *T. viride* at a dose of 150 g per plant also resulted in the lowest tuber rot. Based on Table 2, the percentage of suppression for each observed variable can be calculated. The results of the calculations are presented in Table 3.

Table 3. Suppression of incubation period, intensity, incidence, and tuber rot

Dose (g per plant)	Incubation Period of *S. rolfsii* (%)	Intensity of BPBP (%)	Incidence (%)	Tuber Rot (%)
0	0.00	0.00	0.00	0.00
50	2.19	49.25	58.76	55.14
75	11.50	65.79	65.13	66.28
100	1.68	59.02	66.99	37.65
125	6.73	62.03	56.01	45.92
150	3.42	69.17	58.76	69.46

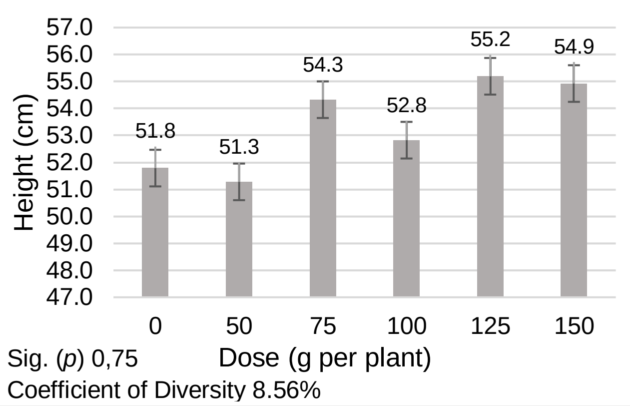


Figure 6. Graph of the effect of various doses of *T. viride* on Porang height

Based on Table 3, it can be concluded that *T. viride* at a dose of 75 g per plant can increase the incubation period of *S. rolfsii* by 11.50%. *T. viride* at a dose of 150 g per plant provides the best suppression of BPBP intensity, at 69.17%, and a suppression of tuber rot percentage at 69.46%. The best suppression of incidence is shown by the treatment of *T. viride* at 100 g per plant. The effectiveness of *T. viride* in suppressing BPBP positively impacts the growth and yield of Porang.

3.3. The Effect of *T. viride* on the Growth and Yield of Porang Inoculated with *S. rolfsii*

3.3.1. Plant Height

The analysis of variance results indicate that the dose does not have a significant effect on plant height. In Figure 6, it can be concluded that the treatment of *T. viride* at 125 g per plant shows the best average height, measuring 55.2 cm, while the treatment of 50 g per plant yields the lowest result at 51.3 cm. Although the differences in doses do not lead to significant variations in plant height, these treatments still show higher results compared to the control. This is due to the role of *T. viride* in enhancing plant growth. According to Garnica-Vergara et al. (2016), T. atroviride, commonly known as T. viride, can produce the compound 6-pentyl-2H-pyran-2-one (6-PP), which promotes plant growth by regulating root structure, inhibiting the formation of primary roots, and stimulating the development of lateral roots, thereby enhancing nutrient absorption. Additionally, according to Contreras-Cornejo et al. (2015) *T. viride* can produce abscisic acid (ABA), an important hormone in regulating plant growth and responses to stress. Contreras-Cornejo et al. (2014) reported that *T. viride* produces hormones such as indole acetic acid (IAA), which regulates cell enlargement, cell division, and tissue differentiation, as well as auxins that play a role in root formation.

3.3.2. Tuber Weight

The planting material used in this study consisted of tubers, so before planting and after harvesting, the tubers were weighed to observe any changes. The results of the measurements are presented in Table 4. Based on the analysis of variance, it can be concluded that the differences in doses of *T. viride* applied do not result in significant variations in the increase in the weight of Porang tubers. However, the treatment of *T. viride* at 150 g per plant yielded the highest result at 376.68 g, while the treatment of 50 g per plant resulted in the smallest increase at 247.25 g.

Although the differences in doses do not result in significant variations in the weight of Porang tubers, it can be concluded from Table 1 that the application of *T. viride* at 150 g per plant yields the highest results. This is attributed to optimal plant growth, which leads to optimal tuber formation.

Table 4. The Effect of Various Doses of *T. viride* on the Increase in Porang Tuber Weight

Dose (g per plant)	Initial weight (g)	Weight gain (g)
0	164.15	259.03±75.42
50	251.3	247.25±119.89
75	177.18	349.05±129.45
100	150.93	347.38±117.45
125	194.38	323.40±141.08
150	240.45	376.68±59.48
Sig. (*p*)		0.305
KK (%)		28.72

Table 5. The Effect of Various Doses of *T. viride* on the Weight of Porang Bulbils

Dose (g per plant)	Bulbil weight (g)
0	6.61±2.96
50	8.49±3.57
75	10.47±4.59
100	7.81±1.88
125	13.53±7.36
150	10.97±3.39
Sig.(*p*)	0.274
KK (%)	43.66

Additionally, the dose of *T. viride* at 150 g per plant provides the best suppression of stem base rot intensity, ensuring that plant growth is not hindered and allowing for optimal tuber production. According to Barus et al. (2018), *T. viride* not only acts as a controller of pathogenic fungi but also functions as a soil decomposer and enhances plant resistance by making nutrients and essential elements available for tuber formation in the soil.

3.3.3. Bulbil Weight

Bulbils or katak are parts of the Porang plant that take the form of tubers growing at the base of branches or on leaf stalks. Bulbils are irregularly round, brown in color, and textured. When cut open, they reveal a bright yellow interior. Bulbils can be used as planting material. Based on the analysis of variance in Table 5, it can be concluded that the differences in doses of *T. viride* do not have a significant effect on the average weight of Porang bulbils.

The average weight of Porang bulbils was smallest in the control group, at 6.61 g, while the largest weight was observed at 13.53 g with the treatment of 125 g per plant. The results for bulbils are closely related to environmental factors and internal plant factors. When soil conditions are fertile, they support the vegetative growth of Porang. This optimizes photosynthate, which is then allocated for bulbil formation. According to research by Halifu et al. (2019), the inoculation of *Trichoderma* sp. can enhance soil nutrient content and enzyme activity in the rhizosphere soil. Inoculation of *Trichoderma* sp. significantly affects the fungal community structure in the rhizosphere soil. It can also improve root length, root area, root diameter, and the number of root branches in plants, thereby increasing the absorption area and promoting seedling growth.

4. Conclusion

The treatment with *T. viride* can enhance plant height and the yield of Porang that has been inoculated with *S. rolfsii*. *T. viride* at a dose of 75 g per plant can increase the incubation period of *S. rolfsii* by 11.50%. *T. viride* at a dose of 150 g per plant provides the best suppression of BPBP intensity at 69.17% and a suppression of tuber rot percentage at 69.46%. The best suppression of incidence is shown by the treatment of *T. viride* at 100 g per plant.

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