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The Effectiveness of the Granular Formulation of a Combination of *Trichoderma sp*. and Bandotan Leaf Extract Against *Sclerotium rolfsii* on Porang (*Amorphophallus oncophyllus*)

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Abstract

Background: *Amorphophallus* oncophyllus is a tuber plant that is beneficial for various industrial sectors, but in its cultivation, it is often affected by the pathogen Sclerotium rolfsii, requiring control measures. Control efforts can be carried out using a combination of the biological agent *Trichoderma sp.* and the plant pesticide made from bandotan leaf extract. The combined control application can be formulated in a solid granular form. **Method:** This research was conducted both in vitro and in vivo. In vitro, a Completely Randomized Design (RAL) was used with the combination of *Trichoderma sp*. and bandotan leaf extract, divided into four levels: 0% (TB0), 5% (TB5), 10% (TB10), and 15% (TB15). In vivo, a 2-factor RAL was applied. The first factor was the concentration of the rice flour carrier material in four levels: 0% (B1), 25% (B2), 50% (B3), and 75% (B4). The second factor was the storage duration of the granular formulation, consisting of 0 weeks (M1), 3 weeks (M2), and 5 weeks (M3). **Result:** The study's results showed that the granular combination of *Trichoderma sp*. and 10% bandotan leaf extract reduced *S. rolfsii* infection by 64.25% and stimulated the growth of porang plant height. **Conclusion:** The treatment with the granular combination of *Trichoderma sp*. and 10% bandotan leaf extract, adding 25% rice flour and a storage period of 0-3 weeks, showed the best effect on the porang plant seedlings.

Keywords: Bandotan leaf; Combined control; Granular; Porang

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Introduction

The porang plant (*Amorphophallus oncophyllus*) is a tuber plant that belongs to the Araceae family. The tubers of the porang plant contain glucomannan, which has many benefits for various industrial sectors, such as raw materials for cosmetic products, adhesives, and the pharmaceutical industry. Therefore, porang tubers have a high economic value. According to the Agricultural Quarantine Agency, the export of porang plants can reach 254 tons, with an export value of 11.31 billion. This figure does not include the domestic demand for porang, for example, in East Java, which requires 600 to 1,000 tons of porang. Therefore, proper cultivation of porang plants is necessary to ensure that production can meet the demand (Rahayuningsih, 2020; Utami, 2021).

The production of porang plants is often hindered by attacks from the pathogenic fungus *Sclerotium rolfsii*, which causes basal stem rot disease in porang plants. Control of the *S. rolfsii* pathogen can be carried out using the biological agent *Trichoderma sp.* (Soenartiningsih et al., 2014), Plant-based pesticide from bandotan extract (*Ageratum conyzoides*) (Isnaini et al., 2021), or with a combination of both, while the use of chemical

pesticides is limited to avoid negative impacts on the environment and the porang plant ecosystem (Ishak & Daryono, 2020). Several plant-based pesticides have been proven effective with biological agents (Djaenuddin & Muis, 2017). Sriwati et al. (2012) Combining *Trichoderma sp*. with several plant leaf extracts has shown promising results. The best result was observed with the combination of *Trichoderma sp*. and 5% bandotan leaf extract, which effectively inhibited the growth of pathogen colonies in vivo. Bandotan leaf extract contains secondary metabolite compounds such as tannins, alkaloids, saponins, triterpenoids, flavonoids, carbohydrates, and proteins, which are beneficial as biopesticides (Kartika et al., 2013). These compounds are consistent with the chemical substances found in Piper betel (red betel) leaves and have been proven to act as toxic compounds for controlling plant pests and diseases (Nugraheni et al., 2020).

The combined control can be applied in a solid granular formulation. In addition to controlling plant diseases, this combined control application can also function as an effective biofertilizer to support plant growth (Affandy et al., 2019). Based on the research by Fazil et al. (2018), the combination of *Trichoderma harzianum*, katuk leaves, and rice bran in a solid pellet formulation was able to reduce *Fusarium sp*. attacks by 33% on tomato plants. Furthermore, Pulungan et al. (2016) showed that combining Trichoderma harzianum and rice flour in a solid granular formulation could control the growth of *Rigidoporus microporus* by 5.55% on rubber plants. Based on the results of these studies, it can be concluded that the biological agent *Trichoderma sp*., when combined with plant extracts containing chemical compounds such as tannins, alkaloids, saponins, and flavonoids, will be compatible and can be used as a control for plant disease-causing pathogens. Therefore, this study aims to evaluate the efficacy of the combination of *Trichoderma sp*. and bandotan leaf extract in a solid granular formulation against the pathogenic fungus *Sclerotium rolfsii*, which causes basal stem rot disease, and its effects on the growth and development of porang (*A. oncophyllus*) plants.

Methods

This research was conducted from February to October 2023 at the Biological Agents Laboratory and Greenhouse of the UPT Plant Protection for Food Crops and Horticulture in Surabaya, East Java. The equipment and materials used in this study include a granulator machine, autoclave, Laminar Air Flow, Petri dishes, Erlenmeyer flasks, micropipettes, hemocytometer, microscope, cork borer (0.5 cm), Potato Dextrose Agar (PDA) media, *Trichoderma sp*. isolates, *Sclerotium rolfsii* isolates, bandotan leaves, Madiun 1 variety porang seedlings, soil, compost, tapioca flour, rice flour, polybags, and 60% paranet.

The in vitro experimental design was conducted on PDA media in the laboratory, using a single-factor Completely Randomized Design (RAL) with the combination of *Trichoderma sp*. and bandotan leaf extract at four concentrations: 0% (TB0), 5% (TB5), 10% (TB10), and 15% (TB15). Each treatment was repeated 5 times, resulting in 20 experimental units. The in vivo experimental design was conducted on porang seedlings in a greenhouse using a two-factor factorial RAL. The first factor was the concentration of the rice flour carrier material, denoted by the letter (B), consisting of 4 levels: 0% (B1), 25% (B2), 50% (B3), and 75% (B4). The second factor was the storage duration, denoted by the letter (M), with three storage periods: 0 weeks (M1), 3 weeks (M2), and 5 weeks (M3). Combining both factors resulted in 12 treatment combinations with three replications, giving 36 experimental units.

Observation parameters included the synergy between *Trichoderma sp*. and bandotan leaf extract, the combination's inhibition capacity, the granular formulation's efficacy, and the growth of porang plant height. Data analysis was performed using IBM SPSS 22, including ANOVA and the Honest Significant Difference (HSD) test at a 5% significance level.

Extraction of Bandotan Leaves

The preparation of bandotan leaf extract is done using water as a solvent. The bandotan leaves are obtained from a bandotan plantation in the Ngoro District, Jombang Regency, East Java. 100 g of bandotan leaves are thoroughly washed and then soaked in 70% alcohol for one minute to kill any microorganisms on the leaves. Next, the leaves are rinsed with distilled water three times to remove the alcohol and then air-dried. After that, 100 ml of distilled water is added to the leaves, and the mixture is blended until smooth. The resulting bandotan leaf solution is then heated for 15 minutes and allowed to steep overnight to ensure maximum extraction of the chemical compounds from the leaves (Sriwati et al., 2012). Finally, the bandotan leaf solution is filtered using a 20-25 µm pore size filter paper to separate the residue and obtain the extract (Khabita et al., 2022; Wiratno et al., 2013).

Synergy Test

The synergy test is conducted to determine the interaction between two combined fungicides. If there is synergy, both fungicides can be applied together, enhancing their toxicity toward pathogen growth. The synergy test uses the poisoning method on PDA media by mixing bandotan leaf extract into the PDA media according to the concentration levels. The concentrations are as follows:

- 0% concentration represents 100 ml of PDA media without bandotan leaf extract
- 5% concentration represents 95 ml of PDA media mixed with 5 ml of bandotan leaf extract
- 10% concentration represents 90 ml of PDA media mixed with 10 ml of bandotan leaf extract
- 15% concentration represents 85 ml of PDA media mixed with 15 ml of bandotan leaf extract

Next, the PDA media mixture is plated into Petri dishes and left to solidify. Afterward, *Trichoderma sp*. is inoculated into the test media by placing a piece of fungal mycelium on the surface of the PDA media. The test isolate is then incubated at room temperature for 4 days. The synergy test is observed macroscopically, including measurements of colony diameter and color, with the expectation that no inhibition of growth or changes in the characteristics of *Trichoderma sp*. occurs after the addition of bandotan leaf extract. (Khabita et al., 2022; Sriwati et al., 2012).

Antagonistic Test

The antagonistic test is conducted to determine the inhibitory effect of the fungicide combination against the pathogen. *Trichoderma sp*. and *S. rolfsii* isolates were obtained from soil exploration in a banana plantation in Ngoro District, Jombang Regency, East Java. The antagonistic test against the *S. rolfsii* pathogen was carried out on PDA media using the dual culture method with the sound diffusion (healthy diffusion) technique.

The first step is plating the PDA media with concentrations of 0%, 5%, 10%, and 15% on Petri dishes, then letting the media solidify. Next, two wells with a diameter of 0.5 cm were made using a cork borer, with a 4 cm distance between them. Each well was inoculated with 20 µl of *Trichoderma sp*. solution and 20 µl of *S. rolfsii* solution. Then, the test isolates were incubated at room temperature for 7 days, and data was collected daily (Aminnullah et al., 2020). The antagonistic test observations include monitoring the antagonistic mechanisms and calculating the percentage of inhibition using the following formula (Nirwanto & Mujoko, 2009):

$$P = (R1 - R2) / R1 \times 100\%$$

P = Inhibition percentage (%)
R1 = Radial growth of the pathogen in control (mm)
R2 = Radial growth of the pathogen in treatment (mm)

Table 1. Categories of Inhibition Percentage (Prastya et al., 2014)

Inhibition Value	Category
> 40%	Strong
40% < x > 30%	Moderate
< 30%	Weak
0%	No antagonistic ability

Combination Granulation

The preparation of the combination granular formulation uses materials such as 100 g of carrier material (compost), 200 g of binder material (tapioca flour), 100 ml of active ingredients (*Trichoderma sp*. and bandotan leaf extract), and an additional carrier material (rice flour) according to the treatment levels. The concentration of bandotan leaf extract used is the extract that provides the best inhibition results against *S. rolfsii* in the antagonistic test.

The first step involves placing all the dry ingredients into the granulator machine and then turning the machine on. Next, the active ingredients (*Trichoderma sp*. and bandotan leaf extract) are sprayed using a hand sprayer until small granular particles are formed. The spraying of active ingredients is done evenly and gradually to prevent the formation of large granule clumps. The formed granules are then air-dried and sieved through a 1 cm mesh to select the appropriate granule size. For the storage treatments, the granules of each treatment are stored in sealed containers to prevent contamination (Affandy et al., 2019; Fazil et al., 2018)

Combination Granular Efficacy

The combination granular application was conducted on porang seedlings aged 28 days after planting (dap). The porang seedlings were obtained from porang farmers in Panglungan Village, Wonosalam District, Jombang Regency, East Java. The growing media used was a mixture of soil and sand in a 2:1 ratio, where porang plants thrive in loose, well-drained soil. The ever-increasing media mixture was sterilized first by oven treatment for 3 days. Inoculation of the *S. rolfsii* pathogen suspension was performed on the seedlings 7 days after transplanting (DAT), and applied to the surface near the base of the porang seedlings' stems.

The combination granular application was made 3 days after inoculation (dai) by placing 15 g of the combination granular near the base of the seedlings' stems in each polybag, according to the treatment. The plants were maintained for 8 weeks. Efficacy observation of the combination granular involved calculating the intensity of the base stem rot disease caused by *S. rolfsii* using the following formula (Munawara & Haryadi, 2020):

$$IP = \Sigma (n \times v) / (Z \times N) \times 100\%$$

IP = Disease intensity (%)
n = number of plants in each disease severity category
v = Scale value for each disease severity category
Z = Scale value of the highest severity category
N = Total number of plants observed

Table 2. Scale for Assessing Plant Damage Caused by Pathogen (Chairudin et al., 2018)

Scale Value	Plant Condition
0	No Damage
1	Light attack, Spots at the base, no wilting
2	Severe attack, spots, wilting, some plants still growing
3	Very severe attack, complete wilting, and plant death

Result and Discussion

The synergy between Trichoderma sp. and Bandotan Leaf Extract

The results of the synergism test (Figure 1) at concentrations of 0%, 5%, and 10% of bandotan extract showed normal growth of *Trichoderma sp*. isolates, with a colony diameter of 9 cm at 4 days after inoculation (dai). The fungal colonies were dark green (5G 3/4), with thick mycelium and distinct colony borders. In contrast, at a concentration of 15%, *Trichoderma sp*. isolates exhibited inhibited growth, with a colony diameter of 9 cm at four dai. The fungal colonies were dark green approaching black (5G 2/1), with thinner mycelium and irregular mycelial borders (Munsell, 1970).

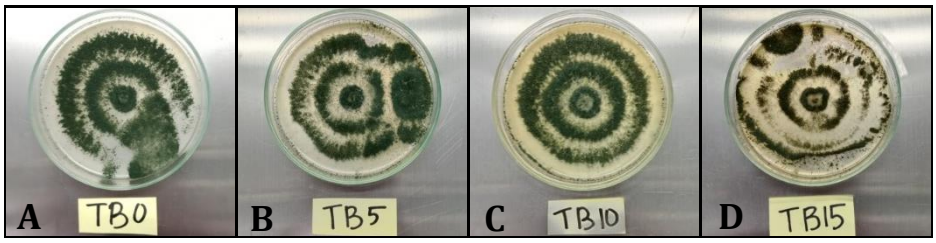

Figure 1. Results of Synergy Test at 4 DAI: (A) Concentration 0%, (B) Concentration 5%, (C) Concentration 10%, (D) Concentration 15%.

This indicates that at concentrations of 5% and 10%, the combination of *Trichoderma sp*. and bandotan leaf extract is compatible and can synergize effectively. In comparison, at 15%, the combination shows less compatibility and synergy. The observed synergy between *Trichoderma sp*. and bandotan leaf extract is closely related to the chemical compounds present in the extract. According to Kartika et al. (2013), bandotan leaf extract contains secondary metabolites such as tannins, alkaloids, saponins, triterpenoids, flavonoids, carbohydrates, and proteins. The carbohydrate and protein content in bandotan extract is thought to provide additional nutrients utilized by *Trichoderma sp.* to stimulate its growth. However, excessive concentrations of these compounds may inhibit the growth of *Trichoderma sp.*, as some of the chemical compounds in bandotan leaf extract have antifungal properties and may cause antagonistic activity between the two. This aligns with Sriwati et al. (2012), who stated that triterpenoid compounds in plant extracts can be used as biopesticides.

Inhibition Power of the Combination

The analysis of variance (ANOVA) results indicate that the combination of *Trichoderma sp*. and bandotan leaf extract significantly affected the inhibition percentage of *Sclerotium rolfsii* (Table 3). This demonstrates that adding bandotan leaf extract concentrations can either enhance or reduce the effectiveness of *Trichoderma sp*. in inhibiting the growth of *S. rolfsii*. The best combination is shown by the treatment with the addition of 10% bandotan leaf extract (TB10), which inhibited the growth of *S. rolfsii* by 32%, placing it in the moderate inhibition category (Prastya et al., 2014).

Table 3. Average Percentage of Inhibition of *Trichoderma sp*. and *Ageratum conyzoides* (Bandotan) Leaf Extract Combination Against *Sclerotium rolfsii* at 7 Days After Inoculation (hsi)

Treatment (Concentration)	Average Percentage of Inhibition (%)
Trichoderma + Bandotan 0%	15% ^{ab}
Trichoderma + Bandotan 5%	22% ^{ab}
Trichoderma + Bandotan 10%	32% ^{b}
Trichoderma + Bandotan 15%	3% ^{a}

Explanation: The same letters in the column indicate no significant difference based on the Honest Significant Difference (HSD) test at the 5% level.

In their study, *Trichoderma sp*. can break down organic materials in the form of complex compounds and utilize them to accelerate growth, thus inhibiting the growth of pathogens. One of the compounds produced by bandotan is tannin, a complex form of proteins, starch, cellulose, and minerals. The availability of this compound can serve as a carbon source for the growth of *Trichoderma sp*. (Kartika et al., 2013).

Antagonistic Mechanism

The results of the antagonistic mechanism observation from the combination of *Trichoderma sp*. and bandotan leaf extract showed parasitism (Figure 2). The hyphal morphology of *S. rolfsii* showed damage, characterized by a reduction in the size of the hyphae due to the treatment with *Trichoderma sp*., where the size of the hyphae differed from the standard hyphal size. This indicates that an antagonistic parasitic mechanism occurred between *Trichoderma sp*. and *S. rolfsii*, in which the hyphae of the antagonistic agent entangled the hyphae of the pathogen. According to Simbolon (2016), The parasitism mechanism occurs when the antagonistic agent entangles the pathogen's hyphae and then releases enzymes such as glucanase and chitinase, which can damage the pathogen's cell wall, leading to the inhibition of the pathogen's growth. Harni et al. (2017) in their study mentioned that *Trichoderma sp*., as an antagonistic agent, produces various secondary metabolites, including protease, cellulase, chitinase, and 1,3-β-glucanase, which play an essential role in plant pathogen control (Dubey et al., 2011).

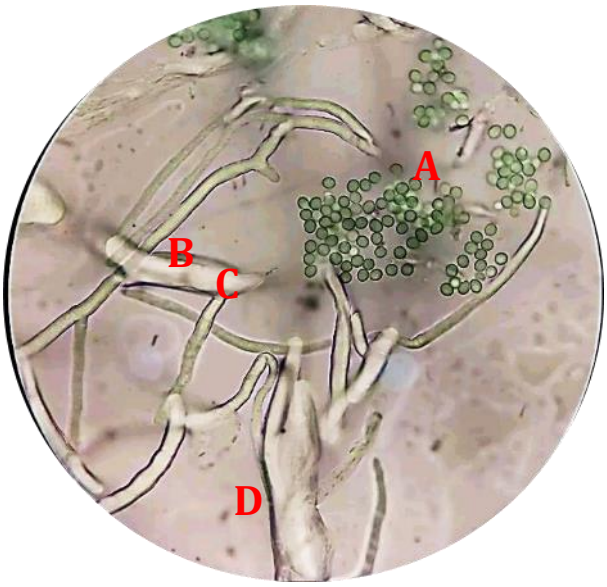

Figure 2. Parasitic Antagonistic Mechanism (400x): (A) *Trichoderma sp*., (B) Hyphal entanglement of *S. rolfsii* by *Trichoderma sp*., (C) Shrinking of *S. rolfsii* hyphae, (D) Normal *S. rolfsii* hyphae.

Efficacy of Combined Granular on Porang Seedlings

The variance analysis results show no significant interaction between the treatment of carrier material concentration and the storage duration of the granules on the average disease intensity. The single treatment of storage duration of the granules showed a very significant effect, while the single therapy of carrier material concentration had no significant effect (Table 4).

This indicates that the granules' storage duration can reduce its active ingredient's effectiveness in controlling and suppressing the attack of the pathogen *Sclerotium rolfsii* on the host plants. The decrease in the efficacy of the active ingredient *Trichoderma sp*. is suspected to occur due to the lack of available nutrients in the combined granular formulation, which causes a decrease in fungal activity. As a result, the fungus does not undergo reproduction, reducing its effectiveness as an antagonistic agent. The *Trichoderma sp*. fungus will enter a dormant phase if the nutrients are insufficient. This

aligns with the statement of Berlian et al. (2013), which suggests that the antagonistic fungus *Trichoderma sp*. tends to form a dormant phase (chlamydospores) to survive in environments that are less supportive of its growth, such as in nutrient-poor conditions.

Table 4. Average Percentage of Collar Rot Disease Intensity on Porang Seedlings with Granular Storage Treatment

Treatment	Average Disease Intensity (%)					
	3 MST	4 MST	5 MST	6 MST	7 MST	8 MST
0 Weeks (M1)	0,00 a	1,00 a	3,17 a	3,50 a	3,75 a	4,42 a
3 Weeks (M2)	0,50 a	3,58 a	5,67 a	9,33 a	12,50 a	13,67 a
5 Weeks (M3)	21,92 b	48,42 b	59,33 b	64,17 b	65,92 b	68,67 b

Explanation: Letters that are the same within the same column indicate no significant difference in the BNJ test at the 5% level.

Porang Plant Height

The results of the variance analysis showed a significant interaction between the treatment combinations of carrier material concentration and granular shelf life on the height of porang plants at 5, 7, and 8 MST, with a very significant interaction at 6 MST. The combination of 0% rice flour carrier material with 0 weeks of granular shelf life (B1M1) and the combination of 25% rice flour carrier material with 3 weeks of granular shelf life (B2M2), resulted in the highest plant height, which was 24 cm (Table 5).

Table 5. Average Height of Porang Plants at 5 MST, 6 MST, 7 MST, and 8 MST with Treatment of Carrier Material Concentration and Granular Shelf Life

Plant Age	Treatment	Carrier Material Concentration (B)			
	Granular Storage Duration (M)	Rice 0% (B1)	Rice 25% (B2)	Rice 50% (B3)	Rice 75% (B4)
5 MST	0 Weeks (M1)	21 b	19 b	17,33 b	17 b
	3 Weeks (M2)	20,33 b	21,67 b	19,33 b	19,33 b
	5 Weeks (M3)	7,33 a	21 b	16,33 b	4 a
6 MST	0 Weeks (M1)	22,33 c	20,67 c	18,33 c	18,67 c
	3 Weeks (M2)	21,67 c	22,67 c	20,67 c	22,23 c
	5 Weeks (M3)	8 b	22 c	4 ab	0 a
7 MST	0 Weeks (M1)	23 c	20,67 c	18,67 bc	18,67 c
	3 Weeks (M2)	22,33 c	22,67 c	13 c	22,33 c
	5 Weeks (M3)	8 ab	22 c	4 ab	0 a
8 MST	0 Weeks (M1)	24 c	22 c	20 c	19,33 bc
	3 Weeks (M2)	23 c	24 c	14 b	23 c
	5 Weeks (M3)	9 ab	22,67 c	4,33 ab	0 a

Explanation: Letters that are the same within the same column indicate no significant difference in the BNJ 5% test.

This indicates that the B2 granular formulation can provide the nutrients *Trichoderma sp*. requires, allowing it to grow and reproduce within the granular formulation. As a result, after storage periods of 0 weeks (M1), 3 weeks (M2), and 5 weeks (M3), *Trichoderma sp*. remained active in promoting growth or acting as a biofertilizer for the plants. The ability of *Trichoderma sp*. functions not only as a biological agent but also as a biological soil fertilizer, decomposer organism, and plant growth stimulator (Herlina et al., 2016; Pulungan et al., 2016). Furthermore, Nurahmi et al. (2012) mention that *Trichoderma sp*. Fungi can produce growth regulators or

phytohormones such as IAA, gibberellic acid, and auxins, stimulating plant growth. The benefits of these phytohormones during the vegetative phase are crucial, particularly in aspects of apical cell dominance, cell elongation, cell division, and root branching in plants (Astriani & Murtiyaningsih, 2018). Therefore, applying *Trichoderma sp*. to plant roots can stimulate root growth to become longer and more abundant, leading to better nutrient and water absorption ultimately resulting in improved plant growth.

Conclusions

The 10% combination of *Trichoderma sp*. and bandotan leaf extract in a granular formulation reduced S. rolfsii infection by 64.25% and promoted the growth of porang plant height. The granular formulation's 0-3 weeks storage period provided the best effect on porang seedlings.

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Declaration statement

The authors reported no potential conflict of interest.

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