

Controlling *Plasmodiophora brassicae* Wor. Clubroot with a Dry Formulation of *Trichoderma* sp. on Broccoli Plants (*Brassica oleracea* L.)

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Abstrak: Salah satu penyakit yang umum pada tanaman brokoli ialah akar gada yang disebabkan oleh patogen tular tanah (*Plasmodiophora brassicae* Wor.). Salah satu bentuk upaya pencegahan penyakit ini dengan mengaplikasikan formulasi kering *Trichoderma* sp. Penelitian ini bertujuan untuk mengetahui pengaruh pemberian formulasi kering *Trichoderma* sp. dalam mengendalikan penyakit akar gada pada Brokoli. Penelitian ini menggunakan Rancangan Acak Kelompok (RAK) dengan 4 perlakuan yaitu P0 (0 gram/liter), P1 (5 gram/liter.), P2 (7 gram/liter), dan P3 (9 gram/liter) dengan 6 ulangan per perlakuan. Hasil penelitian menunjukkan P3 paling efektif dalam meningkatkan tinggi tanaman, helai daun, panjang daun, lebar daun, berat basah, dan bobot bunga brokoli. Sedangkan P2 merupakan konsentrasi yang paling efektif dalam mengendalikan penyakit akar gada dengan tingkat keparahan penyakit paling rendah yaitu 23,85.

Kata kunci: Formulasi Kering; *Trichoerma* sp.; Brokoli; Akar Gada.

Abstract: One of the common diseases in broccoli plants is clubroot caused by soil-borne pathogens (*Plasmodiophora brassicae* Wor.). One form of prevention of this disease is by applying dry formulation of *Trichoderma* sp. This study aims to determine the effect of giving dry formulation of *Trichoderma* sp. in controlling clubroot disease in Broccoli. This study used a Randomized Block Design (RBD) with 4 treatments, namely P0 (0 grams/liter), P1 (5 grams/liter.), P2 (7 grams/liter), and P3 (9 grams/liter) with 6 replications per treatment. The results showed that P3 was the most effective in increasing plant height, leaf blades, leaf length, leaf width, wet weight, and broccoli flower weight. While P2 was the most effective concentration in controlling clubroot disease with the lowest disease severity level of 23.85.

Keyword: Dry Formulation; *Trichoerma* sp.; Broccoli; Mace Root.

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1. Introduction

Broccoli is a vegetable that helps people get the nutrients they need and is a favorite among many people. It has vitamins A and C, carotenoids, fiber, calcium, folic acid, and glucosinolates, which may help prevent cancer. Broccoli

also has fiber and a mix of Omega-3 and beta-carotene, which can help lower cholesterol and keep blood pressure in check [1].

In Indonesia, the need for broccoli is growing each year, especially from hotels, modern markets, and restaurants [2]. This shows that more broccoli is being used, especially in big cities, which means more needs to be grown. However, increasing broccoli production has some problems, like pests and diseases. The most common disease that affects broccoli is called clubroot, caused by a soil-borne pathogen named *P. brassicae* Wor [3],[4]. This disease is one of the most common, affecting between 46 to 89% of crops [5].

Farmers usually use pesticides to manage pests and diseases, but this is not the best way. Using pesticides can harm the environment by polluting water, soil, and affecting other plants and animals. It can also cause health issues for farmers, like feeling sick, dizzy, or even long-term problems like cancer [6]. Plus, using pesticides makes broccoli more expensive to grow, which leads to higher prices when it's sold [7].

An alternative to pesticides is the use of biological agents [8]. One biological agent that has been commonly used is *Trichoderma* sp. [9]. *Trichoderma* sp. is a microorganism that lives in the soil, naturally attacks and takes nutrients from pathogenic fungi [10]. *Trichoderma* sp. has high antagonistic properties against pathogenic fungi and is beneficial to plants [11] and environmentally friendly [12]. Antagonistic activities include competition [13], parasitism, and the formation of toxins including antibiotics for pathogenic organisms [14]. *Trichoderma* sp. can be used as a biological agent to control soil pathogens in the plant root area [15]. *Trichoderma* sp. produces active chemical compounds that damage the cell walls of fungi, thereby disrupting the growth of soil pathogens.

Trichoderma sp. can be grown first in dry corn media and can be directly applied by pouring it around the plant roots [16]. However, this long process can hinder application by farmers. Therefore, other alternatives are needed, such as the use of dry formulations of *Trichoderma* sp. Dry formulations of *Trichoderma* sp. are a composition of biomass flour (*Trichoderma* sp.) and kaolin carrier material in a ratio of 1:1 [17]. The advantages of dry formulations of

Trichoderma sp. are that they can last longer due to the addition of kaolin [18] and are easier to apply. The application of *Trichoderma* sp. has been proven effective in suppressing clubroot disease *Plasmodiophora brassicae* Wor. in cabbage plants [19]. The purpose of this study was to determine the effect of dry formulations of *Trichoderma* sp. in controlling clubroot disease in broccoli plants (*Brassica oleracea* L.).

2. Research Method

This study employed a quantitative experimental approach utilizing a non-factorial Randomized Block Design. The research took place between August and December 2024 at the Biological Agents Lab and Sustainable Food House Area, which are located within the BBPP Lembang Bandung facility. The tools used included an electric stove, an electric pan, stirring rod, beaker, petri dish, reagent bottle, Bunsen burner, lighter, an ose needle, clear plastic, an Erlenmeyer flask, an autoclave, gas cylinder, scale, staples, pen, label paper, newspaper, sieve, jar, blender, spoon, basket box, plastic funnel, measuring pipette, beaker, chemical rack, measuring cup, polybag, shovel, hoe, micropipette, vortex, hotplate stirrer, magnetic stirrer bar, microscope, hemocytometer, cover glass, laminar air flow, spray bottle, tissue, mask, aluminum foil, plastic wrap, rubber bands, and gloves. The materials used included *Trichoderma* sp., dried corn, kaolin, potatoes, distilled water, 70% alcohol, water, roots infected with *Plasmodiophora brassicae* Wor., rice husk charcoal, manure, and soil.

This study implemented four treatments, each repeated six times. The doses administered were based on the study by Yasintasari et al. (2021) [20], with the following details regarding the research approach:

P0 = 0 gr dry formulation of *Trichoderma* sp./liter

P1 = 5 g dry formulation of *Trichoderma* sp./liter

P2 = 7 g dry formulation of *Trichoderma* sp./liter

P3 = 9 g dry formulation of *Trichoderma* sp./liter

The research process consists of the following stages: 1). Preparation of *Trichoderma* sp. Dry Formulation; 2). Preparation of planting media; 3). Preparation of *Trichoderma* sp. Dry Formulation dosage; 4). Application of *Trichoderma* sp. Dry Formulation; 5). Application of clubroot inoculum; 6). Plant cultivation; and 7). Observation [21]. Observation parameters include measurements such as plant height, leaf width, leaf length, the number of leaves, the wet weight of the broccoli, the weight of the broccoli flower, and the level of disease severity. Observation of the broccoli plants started when they were 7 days old and continued until 72 days after they were planted. The measurements of plant height, leaf width, leaf length, and the number of leaves were taken five times, specifically when the plants were 2 weeks old, 4 weeks old, 6 weeks old, 8 weeks old, and 10 weeks old. At the time of harvest, which occurred 72 days after planting, observations were made on the wet weight of broccoli, the weight of broccoli flowers, and the severity of clubroot disease.

The results were analyzed through a one-factor analysis of variance (ANOVA) using SPSS version 23, with a significance level set at 5% ($\alpha = 0.05$). Further testing was conducted using the Duncan Multiple Range Test (DMRT) at a 5% significance level. Regression tests were also conducted to assess how the dosage affects the parameters.

3. Results and Discussion

Based on the observations, the symptoms of clubroot disease, which is caused by *P. brassicae*, can be categorized into two types: those that are visible above the soil surface and those that occur within the roots. The visible signs above ground include the leaves wilting, starting from the stem and spreading to the leaf blade. Infected plants will look healthy again by the afternoon. Root symptoms involve the roots becoming swollen, which may result in the plant wilting.

- a. The impact of using a dry form of *Trichoderma* sp. on the growth of broccoli plants. (*Brassica oleracea* L.)

There were notable variations in plant height observed between 4 to 8 weeks after planting, differences in the number of leaves were noted at 10 weeks after planting, and changes in leaf length were recorded at both 6 and 8 weeks after planting, while variations in leaf width were observed at 6 weeks after planting. The treatment with P3 concentration yielded the most favorable outcomes among all the tested treatments. The most favorable outcomes for P3 included a plant height of 29.28 cm, a total of 51.16 cm for the number of leaves, a leaf length of 20.86 cm, and a leaf width of 11.18 cm.

Table 1. The impact of using a dried formulation of *Trichoderma* sp. on the growth parameters such as plant height, number of leaves, leaf length, and leaf width.

Treatment	Plant Height (cm)				
	2 WAP	4 WAP	6 WAP	8 WAP	10 WAP
P0	6,10 a	13,90 a	17,05 a	21,16 a	24,60 a
P1	6,55 a	14,85 ab	19,55 ab	23,00 a	24,76 a
P2	6,86 a	16,71 bc	22,35 bc	23,35 a	25,68 a
P3	7,00 a	17,71 c	13,70 c	29,28 b	28,16 a
Treatment	Number of leaves (blades)				
	2 WAP	4 WAP	6 WAP	8 WAP	10 WAP
P0	3,66 a	9,00 a	18,66 a	25,16 a	26,66 a
P1	3,66 a	9,16 a	19,50 a	25,83 a	28,00 a
P2	3,83 a	9,83 a	20,66 a	26,16 a	37,33 ab
P3	4,10 a	10,33 a	21,16 a	30,00 a	51,16 b
Treatment	Leaf Length (cm)				
	2 WAP	4 WAP	6 WAP	8 WAP	10 WAP
P0	3,20 a	7,93 a	9,81 a	14,28 a	15,13 a
P1	3,20 a	7,96 a	10,25 a	15,95 a	17,08 a
P2	3,45 a	8,45 a	12,95 a	20,36 b	17,46 a
P3	3,46 a	9,28 a	17,60 b	20,86 b	19,40 a
Treatment	Leaf Width (cm)				
	2 WAP	4 WAP	6 WAP	8 WAP	10 WAP
P0	2,28 a	6,61 a	8,20 a	10,33 a	9,20 a
P1	2,30 a	6,95 a	9,13 ab	10,51 a	10,08 a
P2	2,45 a	6,75 a	9,73 ab	11,86 a	10,10 a
P3	2,50 a	7,71 a	11,18 b	12,01 a	10,41 a

Description: (1) Numbers followed by the same letter indicate no significant difference based on the 5% DMRT test. (2) WAP (Weeks After Planting)

There was a significant difference in plant height between 4 and 8 weeks after planting, but at 2 weeks and 10 weeks after planting, the plant height did not show much variation (Table 1). Observations at 8 WAP indicated that the concentration of P3 was the most effective among all treatments, and the results were significantly different from those of P0. The higher dose of *Trichoderma* sp. used resulted in a significant increase in plant height at 4, 6, and 8 weeks after

planting, with the relationship being directly proportional ($F_{1,24}$ values ranged from 6.702 to 13.535, and p -values ranged from 0.001 to 0.017). There was no significant difference between the treatments observed at 2 and 10 weeks after pollination. In general, the increase in the amount of dry *Trichoderma* sp. formulation applied during weeks 2 and 10 did not show a direct relationship with the growth in plant height ($F_{1,24} = 0.838$, $p = 0.370$ for 2 WAP; $F_{1,24} = 0.406$, $p = 0.531$ for 10 WAP).

The number of leaves showed significant differences only at 10 WAP, whereas at 2-8 WAP, the number of leaves did not differ significantly (Table 1). At 10 weeks after planting, it was observed that the treatment with the highest concentration of P3 yielded the best results, with a leaf length of 51.16 cm, which was significantly greater than that of P0, which measured 26.66 cm. This indicates that the increase in the dosage of the dry *Trichoderma* sp. formulation was significantly associated with an increase in leaf length, as supported by the statistical analysis ($F_{1,24} = 4.390$, $p = 0.048$). Administering the highest dose of *Trichoderma* sp. also demonstrated a positive impact on vegetative growth, generative development, and plant production outcomes [22], as well as contributed to an increase in the number of leaves on the plants [23]. Observations recorded between 2 to 8 weeks after planting showed no significant differences across all the treatment groups. In general, increasing the dose of the dry *Trichoderma* sp. formulation between 2 to 8 weeks after planting did not result in a direct correlation with the number of leaves, as indicated by $F_{1,24}$ values ranging from 0.000 to 3.134 and p -values ranging from 0.091 to 2.140.

The length of the leaves showed significant differences only at 6 and 8 weeks after planting, whereas at 2, 4, and 10 weeks after planting, the differences were not statistically significant (Table 1). Observations at 8 WAP indicated that the concentration of P3 yielded the best results among all treatments, with the highest value of 20.86 cm, which was significantly different from P0, which had a result of 14.28 cm. The higher the dose of *Trichoderma* sp. used, the more the leaf length increased, as shown by the statistical analysis

($F_{1,24} = 15.564$, $p = 0.001$ for 6 WAP; $F_{1,24} = 10.791$, $p = 0.003$ for 8 WAP). Observations made at 2, 4, and 10 weeks after planting did not show any significant differences across all the treatments applied. In general, raising the amount of dry *Trichoderma* sp. formulation applied during weeks 2, 4, and 10 MST did not result in a direct increase in leaf length. The statistical analysis showed that the variation in leaf length was not significantly influenced by the dosage changes, as indicated by F-values ranging from 0.003 to 2.358 and p-values ranging from 0.139 to 0.956.

The width of the leaves showed significant variation only at the 6 WAP stage, whereas at the 2, 4, 8, and 10 WAP stages, the differences in leaf width were not statistically significant (Table 1). At 6 WAP, the observation indicated that the P3 treatment had the highest concentration, with a measurement of 11.18 cm, which was significantly higher than the P0 treatment, which recorded 8.20 cm. Based on the test that was conducted, increasing the dose of the dry formulation of *Trichoderma* sp. during the observation period of 2 to 10 WAP did not result in a direct proportional increase in leaf width. The $F_{1,24}$ values ranged from 0.001 to 4.039, and the p-values ranged from 0.072 to 0.981.

- b. The impact of using a dry form of *Trichoderma* sp. on the wet weight, flower weight, and the level of broccoli (*Brassica oleracea* L.) disease severity.

There was not a big difference in the wet weight and the weight of the broccoli flowers, as shown in Table 2. Studies show that as the amount of *Trichoderma* sp. used increases, the fresh weight and the weight of the flowers in broccoli also go up. There were big differences in how severe the disease was between the treatments, and the least severe case was seen at P2, with a clear and significant difference compared to P0. So, it can be said that increasing the amount of the dry *Trichoderma* sp. formulation does not lead to a direct increase in the wet weight or flower weight of broccoli plants, as shown by the results ($F_{1,24} = 1.512$, $p = 0.232$) and ($F_{1,24} = 1.910$, $p = 0.181$). However, it does lead to a direct decrease in the severity of disease in broccoli plants, as shown by ($F_{1,24} = 4.417$, $p = 0.047$).

Table 2. Effect of Dry Formulation of *Trichoderma* sp. on Wet Weight, Broccoli Flower Weight and Disease Severity

Treatment	Broccoli Wet Weight (gram)	Broccoli Flower Weight (gram)	Disease Severity
P0	14,23 a	4,76 a	41,82 a
P1	161,43 a	10,4 a	29,61 ab
P2	167,2 a	18,66 a	23,85 ab
P3	243,7 a	24,91 a	27,28 b

Description: Numbers followed by the same letter indicate that they are not significantly different based on the 5% DMRT Test.

The wet weight and flower weight of the broccoli plants were measured when they were harvested, which was at 72 hours after seedlings were transplanted. Research using the P3 concentration produced the best results among all the treatments, yielding 243.2 grams, which wasn't significantly different from P0, which had a result of 14.23 grams. The rise in plant fresh weight from treatments P0 to P3 is probably because *Trichoderma* sp. makes growth hormones like auxin IAA, which helps the plant grow more lateral roots and produce shoots [24]. *Trichoderma* sp. is a type of filamentous fungus that thrives in moderate temperatures, is not harmful, and can break down cellulose and hemicellulose into simpler sugars like glucose and xylose. It is commonly used to produce cellulase enzymes, which help in breaking down plant material and increasing plant biomass [25].

The results showed that as the amount of *Trichoderma* sp. increased, the weight of broccoli flowers also increased, but the difference between the treatments wasn't large enough to be considered significant. The P3 concentration gave the best results among all treatments, with a measurement of 24.91 cm. This result wasn't significantly different from P0, which had a measurement of 4.76 cm. The lighter weight of broccoli flowers at P0 is believed to be due to swollen roots that are infected by the *P. Brassicae* pathogen. When plants get infected, they start showing signs below the ground. Their roots become big, twisted, and droopy. These changed roots are usually the first sign you see on the plant's surface when clubroot is present. Infected plants may appear healthy again in the afternoon, which is a sign that they are affected by clubroot disease, a condition caused by the fungus *P. brassicae* [26]. Severely

infected plants will grow slowly, produce low-quality crops, and might die before they are ready to harvest.

4. Conclusion

Using a dry form of *Trichoderma* sp. on broccoli plants (*Brassica oleracea* L.) has been shown to help the plants grow more during their vegetative stage. When you give more *Trichoderma* sp., the plant gets taller, has more leaves, and each leaf becomes wider and longer. *Trichoderma* sp dry formulation was also found to work well in managing *P. Brassicae clubroot* disease in broccoli plants, and it was most effective in treatment P2 ($F_{1,24} = 4.417$, $p = 0.047$).

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