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Dry Preparation of *Trichoderma* Species Promotes Significant Decrease of *Fusarium oxysporum* in Shallot Cultivation (*Allium ascalonicum*)

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Abstrak: Bawang merah merupakan tanaman hortikultura yang mudah terserang patogen. Penggunaan fungisida non-hayati secara berkelanjutan memberikan dampak negatif bagi lingkungan, sehingga perlu adanya pengendalian hayati menggunakan mikroorganisme antagonis. Penelitian ini bertujuan mengevaluasi perbedaan pertumbuhan dan hasil produksi bawang merah yang diberi formulasi kering *Trichoderma* sp. untuk mengendalikan layu *fusarium*. Penelitian menggunakan Rancangan Acak Kelompok (RAK) dengan 4 perlakuan dan 6 ulangan. Konsentrasi yang digunakan yaitu 0 gram/liter, 7 gram/liter, 14 gram/liter, dan 21 gram/liter. Hasil analisis data menunjukkan formulasi kering *Trichoderma* sp. dengan konsentrasi 21 gram/liter memiliki pengaruh paling efektif untuk mengendalikan layu *fusarium* dengan tingkat keparahan serangan paling rendah 23.16 ($F_{1,24}$=47.61, p<0.05) dan secara signifikan meningkatkan tinggi tanaman ($F_{1,24}$=6.36, 7.89, 14.24, p<0.05), jumlah daun ($F_{1,24}$=15.62, 16.07, 18.29, p<0.05), jumlah umbi ($F_{1,24}$=13.57, p<0.05), dan bobot brangkasan ($F_{1,24}$=57.34, p<0.05). Semakin tinggi konsentrasi formulasi kering *Trichoderma* sp. yang diberikan, maka semakin meningkat hasil vegetatif dan generatif tanaman bawang merah.

Kata kunci: *Allium ascalonicum*; *Fusarium oxysporum*; hasil produksi; pertumbuhan; *Trichoderma* sp..

Abstract: Shallot is a horticultural plant that is susceptible to pathogen attacks. The continuous use of non-biological fungicides has a negative impact on the environment, making it necessary to implement biological control using antagonistic microorganisms. This study aims to evaluate the differences in growth and yield of shallots treated with a dry formulation of *Trichoderma* sp. to control Fusarium wilt. The study was conducted using a Randomized Block Design (RBD) with four treatments and six replications. The concentrations used were 0 grams/liter, 7 grams/liter, 14 grams/liter, and 21 grams/liter. The data analysis results showed that the dry formulation of *Trichoderma* sp. at a concentration of 21 grams/liter was the most effective in controlling Fusarium wilt, with the lowest disease severity of 23.16 ($F_{1,24}$=47.61, p<0.05). It also significantly increased plant height ($F_{1,24}$=6.36, 7.89, 14.24, p<0.05), the number of leaves ($F_{1,24}$=15.62, 16.07, 18.29, p<0.05), the number of bulbs ($F_{1,24}$=13.57, p<0.05), and biomass weight ($F_{1,24}$=57.34, p<0.05). The higher the concentration of the dry formulation of *Trichoderma sp.* applied, the greater the increase in the vegetative and generative growth of shallot plants.

Keyword: *Allium ascalonicum*; *Fusarium oxysporum*; growth; *Trichoderma* sp.; yield.

1. Introduction

Shallots (*Allium ascalonicum* L.) are single-season herbaceous plants that are extensively farmed throughout Indonesia. These plants play a crucial role in the, agricultural landscape serving as culinary ingredients, flavorful spices, and an income source for growers. The strong demand in the market positions shallots as a commodity with significant economic worth. Nonetheless, the productivity of shallots can be affected by multiple factors, including environmental contexts, farming methods, and the pressure from pests and diseases. Consequently, investigating methods for growing, managing, and boosting shallot yields is essential in ensuring food supply stability.

The production of shallots in Indonesia from 2021 to 2023 has shown a consistent value, remaining between 1,982,360 and 2,004,590 tons annually [1]. This stability over the past three years is marked by a mere 1.10% variation between the highest and lowest figures, suggesting that there hasn't been marked growth or decline, and production levels fall short of market requirements, leading to ongoing imports. The primary reason for the stagnation in shallot production is primarily due to attacks from pathogens. According to the Ministry of Agriculture's 2023 Data Center, red onion imports increased by 47.78% annually between 2018 and 2022, averaging 632 tons each year (940 thousand USD). Vietnam emerged as the leading source of imports with 512.96 tons, followed by Malaysia at 266.85 tons. The Philippines and Thailand followed with import amounts of 130 tons and 114.58 tons, respectively [2].

One of the common plant pathogens found in shallots is the fungus *Fusarium oxysporum*, responsible for causing moler disease. The first indications that a plant is afflicted by this illness include wilting leaves and the yellowing of leaf tips that may become reddish brown. Symptoms

associated with moler disease involve leaves that appear bent and yellow (chlorosis), and the bulbs may show signs of pale tissue decay [3].

Addressing moler disease can be approached through biological control strategies utilizing antagonistic microorganisms. While biological control is generally more eco-friendly, its effectiveness may not rival that of conventional chemical agents. An example of a biological solution is the fungal species *Trichoderma* sp., which acts antagonistically against *Fusarium oxysporum* and is employed as a biological agent to mitigate *fusarium* wilt in shallots [4].

Trichoderma sp. is frequently found within the soil ecosystem, exhibiting antagonistic properties towards pathogenic fungi, which facilitates the development of robust root systems and promotes deeper root penetration in plants [5]. Trichoderma sp. is a type of soil microorganism that grows in the form of spores, combats harmful fungi naturally, and inhibits the proliferation of other pathogens [6]. Using *Trichoderma* sp. as a biological control agent for managing plant diseases remains relatively straightforward, though it faces challenges due to limited production technology and resources.

The formulation of *Trichoderma* sp. utilized as a biological agent is prepared in a dry format. This dry formulation serves as a biofungicide, made from active *Trichoderma* sp. ingredients, nutritional components that supply carbon and energy for microbial growth, and carrier substances that function as temporary habitats prior to soil application. The dry preparations of T*richoderma* sp. are advantageous for their practicality, ease of field application, and extended shelf life [7].

This research seeks to evaluate the impacts of the dry formulation of *Trichoderma* sp. on plant growth, yield outcomes, and effectiveness in controlling disease. The findings of this investigation aim to offer a sustainable biological control alternative for combating fusarium wilt in shallots.

2. Research Methods

The research method employed involved a quantitative experiment utilizing a non-factorial Randomized Block Design. This investigation took

place between August and December 2024 at the Biological Agent Laboratory and the Sustainable Food House Area of BBPP Lembang, in West Bandung, West Java. For the study, various tools were utilized, including a microscope, haemocytometer, cover slips, digital scales, stirring rods, measuring cups, test tubes, a Laminar Air Flow (LAF), ose needles, autoclaves, sieves, blenders, ovens, meters, and polybags sized at 40×40 cm. The materials involved included corn media, isolates of *Trichoderma* sp., kaolin, spores of *Trichoderma* sp., isolates of *Fusarium oxysporum*, shallot seeds, PSA (Potato Sucrose Agar) media, and growing mediums.

There were four different concentrations tested across six replication, leading to the collection of 24 plant samples. The concentration levels were adapted from the work of Yasintasari & Hadi, with the optimal dosage of *Trichoderma* sp. being set at 7 grams per liter [8]. The four treatment categories implemented in this research comprised: P0 (0 grams per liter), P1 (7 grams per liter), P2 (14 grams per liter), and P3 (21 grams per liter).

a. Preparation of Dry Formulation of Trichoderma sp. and Calculation of Spores

Corn is measured to weigh 1 kg, then thoroughly cleaned and soaked for a period of 12 hours. After soaking, the corn is rinsed, drained, and allowed to dry. Next, the corn medium undergoes sterilization in an autoclave, operating at 121 degrees Celsius under a pressure of 1 ATM for 30 minutes, followed by a cooling phase. The sterilized corn medium is placed into a laminar airflow hood for the inoculation process using *Trichoderma* sp. This inoculation involves taking a 1×1 cm piece of the isolate and blending it with the corn medium. Within 7 days, a culture of *Trichoderma* sp. will be established. The medium is then dried once more, blended, and filtered to separate *Trichoderma* sp. spores from any remaining corn debris. The *Trichoderma* sp. spores are combined with a carrier substance, specifically kaolin, in a 1:1 ratio to create a dry formulation of *Trichoderma* sp.

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The purpose of calculating spore density is to determine the appropriate quantity of fungal spores for field application. One gram of the dry *Trichoderma* sp. formulation is added to a test tube with 0.9 ml of water and vortexed for 3 minutes. A 0.1 ml sample is then carefully placed on to the haemocytometer and covered with a cover slip. Spores are counted in the designated counting chamber under a microscope with a magnification of 400×.

b. Propagation of *Fusarium oxysporum* Isolates

Fusarium oxysporum samples were sourced from the Vegetable Plant Instrument Standard Testing Center (BPSITS), in West Java. Additionally, the isolate underwent a purification process and was expanded on both Potato Sucrose Agar (PSA) and corn media. The approach involved taking 100 grams of the fusarium isolate, introducing it to PSA and corn media, and then allowing it to incubate for a span of 14 days.

c. Preparation of Materials and Planting Media and Inoculation of *Trichoderma* sp.

Using red onion bulbs from the Batu Ijo variety, characterized by their consistent bulb size of 5 grams and being divided into quarters as planting materials. The growing medium comprises ($^1/_2$) manure: (1) part rice husk charcoal: and (1) part soil, which is packed into a polybag measuring 40x40 centimeters. The dry formulation of *Trichoderma* sp. is inoculated by preparing a solution based on the treatment being evaluated and subsequently delivering it to the plants. This application occurs 7 days prior to planting, on the planting day, and again seven days post-planting.

d. *Fusarium oxysporum* Inoculation and Planting and Maintenance

Inoculation with the *Fusarium oxysporum* fungus takes place when the plants have reached 14 days of age. This process involves applying 200 ml of the *Fusarium oxysporum* pathogen, which is prepared by dissolving 160 grams of the fusarium isolate in 6 liters of water, into every planting hole. Each onion bulb is positioned at a depth of 2-3 cm and receives watering every other day, either in the morning or evening. For pest

management, insecticides containing Bacillus thuringiensis are utilized at a concentration of 1 gram per liter.

e. Observation Parameters

The parameters that were examined included the growth height of the plants, leaf count, tuber quantity at the time of harvest, dry stalk mass, and the level of disease affliction. Assessments regarding plant height and leaf number were conducted at intervals of 14 days after planting (HST), 28 days after planting (HST), and 42 days after planting (HST). In contrast, the evaluation of tuber quantity, dry stalk mass, and disease severity took place upon harvesting the plants at 65 HST.

To measure plant height, the distance from the ground to the highest point of growth was recorded using a measuring meter. The leaf count was determined by tallying the number of fully developed leaves. Tuber counting was performed during the harvest phase. The stalk mass was obtained after drying all components for a period of 7 days.

A key parameter that was monitored was the degree of disease severity, which was assessed in the following manner:

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

Where:
IP= Disease intensity (%),
n= Number of symptomatic leaves in each category,
v= Attack category value,
Z= Highest attack category value,
N= Number of leaves observed [10].

Table 1. Disease Severity Categories

Score	Description
0	No symptoms
1	Symptoms of yellowing leaves 0-20%
2	Symptoms of yellowing leaves 21-40%
3	Symptoms of yellowing leaves 41-60%
4	Symptoms of yellowing leaves 61-80%
5	Symptoms of yellowing leaves >80%

f. Data Analysis

The data collected from observations underwent testing through a one-way Analysis of Variance (ANOVA) set at the 5% significance level (α= 0.05). In cases where treatments showed significant differences, the analysis proceeded with the Duncan Multiple Range Test (DMRT) at the 5% threshold. A regression analysis was performed to evaluate how varying concentrations of the dry formulation of *Trichoderma* sp. impacted the five parameters observed.

3. Results and Discussion

a. Plant Height and Number of Leaves

A notable variation in the height of plants was observed at both 14 days after treatment and 28 days after treatment, while no significant changes were seen at 42 days after treatment (Table 2). The application of the P3 concentration (21 grams per liter), yielded the most favorable outcomes among all tested treatments, achieving a maximum height of 40.28 cm, which was significantly greater than the control group (P0), which recorded a maximum height of 28.60 cm. The relationship between the concentration of the dry formulation of *Trichoderma* sp. and plant height exhibited a direct correlation, with results indicating $F_{1,24}$=14.24, $p<0.05$ for 14 days after treatment; $F_{1,24}$=7.89, $p<0.05$ for 28 days after treatment; and $F_{1,24}$=6.36, $p<0.05$ for 42 days after treatment.

Table 2. The Effect of Dry Formulation of Trichoderma sp. on Plant Height

Treatment	Plant Height (cm)		
	14 HST	**28 HST**	**42 HST**
P0	13.717 a	28.600 a	41.617 a
P1	13.983 a	31.183 ab	41.883 a
P2	19.000 ab	37.833 ab	45.800 a
P3	22.483 b	40.283 b	49.517 a
	BN	BN	TBN

Description: Number notation followed by the same letter means no significant difference in Duncan's multiple range test at 5% level.

There was a notable variation in leaf counts at the 14 days after planting (HST), 28 days after planting (HST), and 42 days after planting (HST) (Table 3). The application of the P3 concentration (21 grams/ liter) yielded the best outcomes among all treatments evaluated, achieving an average of 63.67 leaves, which was significantly superior to the P0 (control) treatment, which only produced 30.00 leaves. The increased leaf quantity was directly linked to the incorporation of Trichoderma sp. ($F_{1,24}$=16.07, $p<0.05$ at 14 hours; $F_{1,24}$=15.62, $p<0.05$ at 28 hours; and $F_{1,24}$=18.29, $p<0.05$ at 42 hours).

Table 3. The Effect of Dry Formulation of *Trichoderma* sp. on the Number of Leaves

Treatment	Number of leaves (blades)		
	14 HST	**28 HST**	**42 HST**
P0	14.00 a	23.33 a	30.00 a
P1	17.33 a	26.83 ab	33.50 ab
P2	26.67 b	42.17 bc	51.00 bc
P3	28.50 b	48.33 c	63.67 c
	BN	BN	BN

Description: Numeric notation followed by the same letter means no significant difference in Duncan's multiple range test at 5% level.

Trichoderma species have the ability to enhance the growth of plants. This particular fungus is recognized for its capacity to generate growth hormones like indole acetic acid (IAA), gibberellin, and cytokinin [11]. IAA promotes both root development and lengthening, enabling improved nutrient uptake from the soil [12][13]. The hormones auxin and cytokinin play a significant role in the seed germination process due to their collaborative function during embryo development. Cytokinin triggers cell division while auxin encourages cell elongation [14]. Gibberellin hastens the germination process, boosts seed vitality, and improves the efficiency of red onion seed sprouting [15][16].

Measurements of plant height taken at 42 HST demonstrated fairly consistent findings. This consistency arises because the shallot plants are transitioning into the initial stage of bulb development, moving away from vegetative growth. Additionally, the genetic characteristics of

different shallot varieties influence both the growth in height and the plant's physical traits [17][18].

The management of dry formulations of *Trichoderma* sp. influences the rate at which leaves grow. At 14 days after sowing, the number of leaves increases significantly due to enhanced nutrient absorption [19]. The growth hormones, decomposing enzymes, and bioactive substances generated by *Trichoderma* sp. directly promote both the height growth and leaf count by improving nutrient availability and encouraging the division and development of leaf cells [20].

b. Number of tubers, dry weight of the stalk and severity of the disease

There were notable variations in the quantity of tubers, weight of dry stubble, and severity of disease (Table 4) among the different treatments. Treatment P3, with a concentration of 21 grams/liter, yielded the most favorable outcomes compared to all other treatments applied. In treatment P3, the tuber count reached 8.67, the weight of dry stubble was recorded at 232.73 grams, and the severity of disease was noted at 23.16, all of which were markedly distinct from P0. These three measured parameters showed significantly elevated values when compared to the other three treatments. This indicates that an increase in the concentration of *Trichoderma* sp. correlates notably with a rise in tuber counts ($F_{1,24}$=13.57, $p<0.05$) and dry stubble weight ($F_{1,24}$=57.34, $p<0.05$) and demonstrates the most effective impact against the Fusarium oxysporum fungus ($F_{1,24}$=47.61, $p<0.05$).

Table 4. The Impact of Dry Formulation of Trichoderma sp. on Bulb Quantity, Weight of Dry Stump, and Severity of Disease

Treatment	Number of tubers (fruit)	Dry Stove Weight (grams)	Severity of Disease
P0	3.83 a	28.4467 a	68.1050 a
P1	4.83 ab	112.0667 b	32.8967 ab
P2	8.00 bc	149.9833 b	29.3883 b
P3	8.67 c	232.7333 c	23.1633 c
	BN	BN	BN

Description: Numeric symbols that are succeeded by the corresponding letter indicate there is no meaningful difference found in Duncan's multiple range test at a 5% significance level.

Elevating the concentration of the dry *Trichoderma* sp. formulation led to improved outcomes across all observed parameters [Figures 1 and 2], resulting in enhanced plant yield and more effective defense strategies against *fusarium* wilt infestations. The development of red onion bulbs hinges on the progression of the vegetative stage. The quantity of leaves produced and robust root system correlate with the bulb formation rate [6].

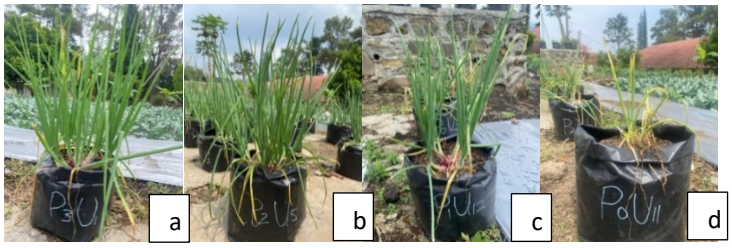

Figure 1. Symptoms of plants infected with *fusarium*: a=P3; b=P2; c=P1; d=P0

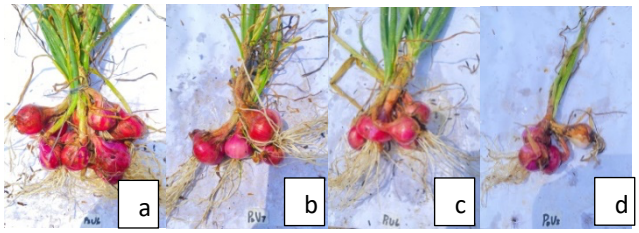

Figure 2. Tuber rot due to *Fusarium* wilt: a=P3; b=P2; c=P1; d=P0

Trichoderma species influence the mass of dry stalks through the organic materials created from photosynthesis and nutrient uptake. This fungus enhances the efficiency of photosynthesis by generating growth-promoting substances, which lead to an increase in leaf quantity and surface area, allowing the plants to absorb more sunlight. An elevation in photosynthesis yields more carbohydrates and energy necessary for metabolic functions, thereby boosting the weight of dry stalks. The enhanced development of root hairs and the overall improvement in root structure owing to the presence of Trichoderma species impact the dry mass of tubers [21].

Trichoderma sp. serve as a biological control method intended to manage diseases that occur after harvest. This competitive fungus acts as a biocontrol agent against diseases affecting various plants [22]. *Trichoderma* sp. operates in

a more natural fashion, as it doesn't directly eliminate disease-causing agents. Instead, it generates antibiotic substances, envelops other harmful fungal filaments, compromises their cellular structures to absorb their nutrients, and competes for the available nutrients [23].

The disease known as *Fusarium* wilt starts to affect shallot plants around 28 days after transplanting, continuing until the time of harvest. The first signs of infection include yellowing curled leaves and tubers that show signs of decay. Additional symptoms consist of deformed leaves, a pale green hue, wilting, impeded growth, and rotting at the base of the stems [24][25].

4. Conclusion

The findings indicated that the powdered form of *Trichoderma* sp. effectively controlled the moler disease triggered by the fungus *Fusarium oxysporum*, while also enhancing the growth and yield of shallots. The rise in the level of *Trichoderma* sp. corresponded strongly with height growth, leaf count, bulb quantity, stalk weight, and a reduction in the intensity of fusarium wilt infections.

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