Application of *Trichoderma viridae* with organic fertilizers as carrier for controlling *Sclerotium rolfsii* disease in chili

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**Abstract.** Stem rot disease caused by *Sclerotium rolfsii* is the main disease in chili plants that is difficult to control. The objectives of this study were: 1] to obtain the type of organic fertilizer that can increase the population of *T. viridae*, 2] to determine its effectiveness in controlling *S. rolfsii* that causes stem rot disease, and 3] to improve the growth of chili plants. This study consisted of two stages, which were mass propagation of *T. viridae* in various organic fertilizers to increase its population before application to the rhizosphere of chili plants and the testing of the effectiveness of *T. viridae* to control stem rot and improve the growth of chili plants. The research was arranged in a Randomized Block Design consisting of four treatments with six replications within treatments. The treatments were various organic fertilizers, including cow manure, chicken manure, crop residue compost, and control. The variables observed were the population of *T. viridae* in each organic fertilizer after 15 days of incubation and the attack rate of chili stem rot [incubation period, disease severity, and plant growth]. The results showed that the crop residue compost was the best medium for increasing the density of *T. viridae* conidia and propagules. Crop residue compost was also the best in suppressing stem rot disease caused by *S. rolfsii*, as well as in increasing the growth of chili plants.

**Keywords:** chili, organic fertilizer, *Sclerotium rolfsii*, *Trichoderma viridae*

1. Introduction

*Sclerotium rolfsii* Sacc is one of the important pathogens causing stem rot disease in chilies. That attack at the nursery and plant stages in the field [1]. *S. rolfsii* attack in endemic areas can reduce production by 25-55% [6]. Disease control in chili plants commonly through the application of pesticides. However, the intensive application results in a negative impact on the environment and consumers [16]. Accordingly, we need another control alternative that is environmentally friendly, namely biological control by using *Trichoderma* spp. fungi as biological control agents.

The results of previous studies indicate that the antagonistic fungi *Trichoderma* spp. are the potential to inhibit the growth of *S. rolfsii*. *Trichoderma* spp. derived from the peanut rhizosphere can inhibit the growth of *S. rolfsii* in vitro and planta. These antagonistic fungi can also infect sclerotia, making them rot and unable to germinate [10; 11]. *T. harzianum* isolates were able to reduce 30-50% [damping-off] in green beans caused by *S. rolfsii* [14].

The effectiveness of *Trichoderma* isolates potentially used to suppress stem rot disease caused by *S. rolfsii* needs to be tested in planta. The application of *Trichoderma* with carrier organic fertilizers will improve its role as a biological control agent for diseases caused by *S. rolfsii* and as a plant growth promoter. Organic matter in the form of plant and animal residue compost contains macro and
micronutrients [13], cow dung manure has high fiber content such as cellulose, macro, and microelements [15]. Crop residue compost of banana containing macro dan microelement and carbohydrate [2].

The purpose of this study was to obtain the best type of organic fertilizer as a carrier for T. viridae to increase its population before applied to the rhizosphere, as well as to determine the effectiveness of T. viridae in controlling stem rot disease in chilies.

2. Materials and Method

2.1. Experimental Design

This experiment used a randomized block design consisting of four treatments with six replications within treatments: cow manure, chicken manure, and compost from banana crop residue, and control with six replications. The data were processed with analysis of variance and further tests using the least significant difference [LSD].

2.2. Research Application

2.2.1. Rejuvenation and Propagation Trichoderma viridae in Organic Fertilizers

Trichoderma viridae was cultured on Potato Dextrose Agar medium for six days. Organic fertilizer was placed on a plastic tray with a diameter of 55 cm, contained 1.5 kg, then was inoculated with T. viridae with a conidia density of 6.10^6 / ml, which was as much as 10% of the weight of the organic fertilizer, incubated for 15 days.

2.2.2. Propagation of Pathogens

Sclerotium rolfsii was cultured on the PDA medium and incubated for six days. For inoculation, S. rolfsii was propagated in a medium mixture of sand and corn starch with a ratio [1: 1] for 14 days.

2.2.3. Soil Preparation and Treatment

The soil used comes from the Limau Manih area. The soil sterilized for an hour using a steamer. 10 kg of the soil was put in a polybag. The treatment was given by mixing T. viridae in organic fertilizer as much as 10% of the weight of the planting medium.

2.2.4. Preparation of Chili Seeds and Planting

The seedlings that the 30-days-old were planted in polybags containing the growth medium treated with T. viridae in organic fertilizer, maintenance: watering, weeding, and fertilization.

2.2.5. Inoculation of Sclerotium rolfsii

The inoculation was carried out 14 days after planting, a hole was made around the base of the chili stems with a depth of 5 cm, and S. rolfsii was sown in the hole.

2.3. Observation

The parameters observed were: a]. The density of Trichoderma viridae, a1] Conidia in the Organic Fertilizers, calculated with Gabriel and Riyanto formula [4], a2] Propagules of Trichoderma viridae in the organic fertilizers calculated with Lenc formula [8], b]. Stem rot disease in chili plants: b1] the incubation period, b2] disease incidence, c] Plant Growth: c1] Plant height, c2] number of leaves, c3] number of branches.

3. Result and Discussion

3.1. The density of T. viridae conidia in various organic fertilizers

The density of T. viridae conidia in various organic fertilizers incubated for 15 days showed significantly different results, which can be seen in Table 1.


### Table 1. The density of *T. viridae* conidia in various organic fertilizers incubated for 15 days

| Treatment                        | The density of *T. viridae* conidia Before application | Treatment                        | The density of *T. viridae* conidia After application |
|----------------------------------|-------------------------------------------------------|----------------------------------|------------------------------------------------------|
| Crop residue compost             | 4.58 x 10^3 a                                        | Crop residue compost             | 5.87 x 10^3 a                                        |
| Cow manure                       | 3.75 x 10^3 ab                                       | Cow manure                       | 4.87 x 10^3 ab                                       |
| Control                          | 3.20 x 10^3 b                                        | Chicken manure                   | 4.67 x 10^3 b                                        |
| Chicken manure                   | 2.74 x 10^3 b                                        | Control                          | 4.58 x 10^3 b                                        |

3.2. *The density of *T. viridae* propagules in various organic fertilizers*

The density of *T. viridae* propagules in organic fertilizers incubated for 15 days before and after application can be seen et [Figure 1].

![Figure 1. The density of *T. viridae* propagules in various organic fertilizers incubated for 15 days](image)

Crop residue compost and cow manure were good substrates for carrying *T. viridae* before applying to the plant rhizosphere. In general, plant and animal waste compost contains macro [N, P, K] and micro [Mg, Mn, Bo, Fe, S, Zn, Co] elements [13]. Crop residue compost from banana containing carbohydrate, macro, and microelement [9] that can be broken down by *T. viridae* as a source of nutrition so that *T. viridae* can grow well. Cow dung manure has high fiber content such as cellulose, NPK, and other microelements [15], making it suitable for the growth of *T. viridae* before application to the plant rhizosphere. *T. viridae* produces cellulolytic enzymes, including cellobiohydrolase, endoglucanase, and β-glucosidase [5].

The number of *T. viridae* propagules before its application to the plant rhizosphere was higher than after application. This result is because *T. viridae* applied for 90 days of the chili plant growth can grow well since it uses organic fertilizers as basic food before it can adapt to the new environment. Furthermore, it can grow and develop optimally. It is because root exudates also influence the growth of *T. viridae*. According to Zhuang et al.,[17] state, that root exudate is the main source of food and energy for organisms living in the rhizosphere.
3.3. The appearance of the first symptoms

The appearance of the first symptoms of S. rolfsii in chili plants with various treatments of T. viridae in various organic fertilizers as carriers showed in Table 2.

Table 2. The appearance of the first symptoms of S. rolfsii causing stem rot disease in chili plants that treated with T. viridae in various organic fertilizers as carriers

| Treatment          | The appearance of the first symptoms [days] | Notes                                                        |
|--------------------|---------------------------------------------|--------------------------------------------------------------|
| Crop residue compost | -                                           | No symptoms observed until the end of observation [7 weeks] |
| Cow manure         | 5.00                                        |                                                              |
| Chicken manure     | 7.00                                        |                                                              |
| Control            | 4.00                                        |                                                              |

3.4. The intensity of Stem Rot Disease in Chili Plants caused by Sclerotium rolfsii

In chili plants treated with the crop residue compost, there were no symptoms observed until the end of the observation. Meanwhile, in chili plants treated with cow manure, the disease intensity was lower than the chicken manure [Figure 2].

![Figure 2](image)

**Figure 2.** The intensity of stem rot disease in chili plants caused by S. rolfsii treated with T. viridae in various organic fertilizers as carriers

The chili plants treated with T. viridae in crop residue compost did not show any symptom of stem rot disease caused by S. rolfsii until the end of the observation. This result is due to the role of T. viridae in suppressing the growth of S. rolfsii after being introduced into the rhizosphere of chili plants. T. viridae incubated in crop residue compost for 15 days had a higher growth compared to that in other treatments both before and after applied to plant rhizosphere [Table 1 and Figure 1]. The conidia and propagules of T. viridae applied with crop residue compost could adapt and develop in the plant rhizosphere. Trichoderma in the rhizosphere of chili plants can inhibit the growth of S. rolfsii by various mechanisms, such as competition, antibiosis, and parasitism, thereby inhibiting the growth of pathogen and preventing disease in chili plants. According to Zhuang et al.,[17] T. viridae released an antibiotic in the form of viridiol phytotoxin, which could inhibit the development of pathogens.
3.5. The Growth of Chili Plants

3.5.1 Plant Height
The tallest plant was found in the treatment of crop residue compost, followed by the plants treated with cow manure. Meanwhile, the treatment of chicken manure resulted in the shortest plants [Figure 3].

![Plant Height Chart]

**Figure 3.** The plant height of chili plants treated with *T. viridae* in various organic fertilizers

3.5.2 Number of Leaves
The highest number of leaves of chili plants was also found in the plants treated with crop residue compost, followed by cow manure and chicken manure [Figure 4].

![Number of Leaves Chart]

**Figure 4.** The number of leaves of chili plants treated with *T. viridae* in various organic fertilizers as carriers

3.5.3 Number of Branches
The highest number of branches was found in the chili plants treated with crop residue compost, followed by the plants treated with cow manure and chicken manure [Figure 5].
Figure 5. The number of leaves of Chili Plants Treated with *T. viridae* in various organic fertilizers as carriers

The growth of chili plants treated with various organic fertilizers as a carrier for *T. viridae* showed that the application of crop residue compost resulted in higher values of all variables compared to other treatments. This result is related to the high population of *T. viridae* after being applied to the plant rhizosphere. It is known that *Trichoderma* can improve plant growth. The application of *T. virens* strain TV.29.8 and *T. atroviride* strain IMI 2060040 could improve root growth in *Arabidopsis* seedlings indicated by an increase IAA in plant tissue [3]. The colonization of *T. viridae* with T1sk isolates on the surface and in the root [endophytic] of banana seedling tissue was able to increase the number of leaves, plant height, and dry weight of plant biomass [12].

4. Conclusion
Crop residue compost was the best medium in increasing the density of conidia and propagules of *T. viridae* before and after applied to the rhizosphere of chili plants. Crop residue compost was also the best in suppressing stem rot disease caused by *S. rolfsii*, as well as in improving the growth of chili plants.

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