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Effects of *Trichoderma* sp. and vermicompost for controlling *Sclerotium rolfsii* in chili pepper crops in tropical peat soil

***Adi Jaya[1], Magdalena Marpaung[2], Lilies Supriati[3], Bambang S. Lautt[4], Emmy Uthanya Antang[5] and Sosilawaty[6]**

Faculty of Agriculture, University of Palangka Raya, Jalan Yos Soedarso, Palangka Raya, Indonesia

Email: magdalenamarpaung38@gmail.com[2], lilies_supriati@yahoo.com[3], blautt63@gmail.com[4], emmyantang@yahoo.com[5], susisibot@gmail.com[6]

*Correspondence email: adijaya_unpar@yahoo.co.id[1]

Corresponding Author

Adi Jaya

Faculty of Agriculture, University of Palangka Raya, Jalan Yos Soedarso, Palangka Raya, Indonesia

**Corresponding Author Email: adijaya_unpar@yahoo.co.id[1]*

Abstract

Control of the fungal disease *Sclerotium rolfsii* (southern blight) is important for increasing productivity and quality of crops of chili pepper (*Capsicum frutescens* L.). Among other measures, this disease can be controlled using biological agents. We examined the influence of fungus*Trichoderma* sp. and vermicompost in controlling *Sclerotium rolfsii*. The study was conducted in laboratory and at experimental sites of Palangka Raya University, Indonesia. A Factorial Complete Randomized Design was used, with four levels of *Trichoderma* sp., and with three levels of vermicompost. Observations included incubation period, disease attack intensity, Number of sclerotial (sclerotia), *Sclerotium* germination, antagonistic effectiveness, and observation of the microbial population. Results showed that there was an interaction effect of application of *Trichoderma* sp. and vermicompost in controlling the fallow disease phase of *Sclerotium rolfsii*. A combination of *Trichoderma* and vermicompost affected the length of the incubation period during which no disease symptoms were observed. Control effectiveness pathogenic *Sclerotium rolfsii*, was in the excellent category, with 100% effectiveness value, the highest sclerotia germination occurred in the control treatment, and the lowest percentage, at 47.6%, in the combination of *Trichoderma sp*. treatment 10 gr per box and vermicompost 100 gr per box.

Keywords: *Trichoderma* sp., vermicompost, *Sclerotium rolfsii*, chili pepper.

Introduction

Chili pepper (*Capsicum frutescens* L.) is an important vegetable commodity in Indonesia with a high market demand. However, cultivating chilli from seed often faces obstacles from pests and diseases which can have a negative impact the quality and quantity of the crop. Often an attack by pathogenic fungi will occur in the early vegetative phase when the plant is no more than four weeks old. This can result in high mortality and, even if plants survive, a 53% decrease in the quantity of the crop yield which is also of lower quality (Ferry and Dukes, 2005). One of the most common fungal pathogens is *Sclerotium rolfsii (*southern blight) which causes several

deadly diseases in plants such as stem rot, wilting and sprouting. This is a soil-borne fungus that can persist for long periods in the form of sclerotia in the soil, manure, and the remnants of diseased plants. In addition, the fungus can spread through irrigation water and on crop land where the pathogen has existing host plants. Differences in the characteristics of the *Sclerotium rolfsii* fungus in some host plants have been observed, including colony diameter, mycelial growth rate, size and color of sclerotia (Magenda *et al*., 2011). This pathogen is difficult to control because it has a wide range of hosts and can survive for many years in the soil in the form of hyphae or sclerotia which are facultatively parasitic and can live as saprophytes if no host plants are available (Semangun, 1993). This type of facultative microorganism has low saprophytic competition ability and so the use of synthetic pesticides is less effective (Sumartini, 2012). In order for crops to enter the global market, there is increasing demand for environmentally friendly agricultural products, i.e. for food that is produced without the use of synthetic pesticides. One alternative approach to crop disease control is the use biological agents as substitutes for synthetic pesticides (Harwitz, 2003 in Tjut et *al*., 2011).

The use of biological control methods can reduce the negative influences on the agricultural environment and its surroundings that are associated with most conventional pesticides. *Trichoderma* sp. is amongst the known fungi that have the potential to act as biological agents of pathogenic fungi (Tindaon, 2008). Besides its role as an antagonist, it is also known that *Trichoderma* sp. functions as a decomposer in making organic fertilizer (Wijaya et *al*., 2012). Thus this fungus can perform two beneficial functions in the soil environment. Baker and Cook (1982) in general the mechanism of antagonism of *Trichoderma* sp. in suppressing pathogens, namely as a micoparasitic and aggressive competitor. Initially, the hyphae of *Trichoderma* sp. grow elongated, then convolute and penetrate the pathogenic fungal hyphae so that the host hyphae are vouleded, lysis and finally destroyed. According to Harjono and Widyastuti (2001), *Trichoderma* sp. penetrate into the host cell wall with the help of cell wall degrading enzymes namely chitinase, glucanase, and protease, then use the contents of the host hyphae as a food source. When twining and producing enzymes to penetrate the host cell wall, *Trichoderma sp*. also produce antibiotics such as gliotoxins and viridian. Papavizas (1985) in Tjut *et al*. (2011) stated *Trichoderma* sp. which is micoparasitic will suppress the population of pathogenic fungi that previously dominated; the interaction begins by wrapping the hyphae on the pathogenic fungus which will form a hook-like structure called the haustorium and align the pathogenic fungus. Along with hyphae stabbing, the mycoparasite fungi secrete enzymes such as the enzyme chitinase and β-1,3glucanase which will destroy the pathogenic fungal cell walls. As a result, the pathogenic hyphae fungus will be damaged, its contents come out and the pathogenic fungus will die. At the same time an antibiotic mechanism occurs, the release of anti-fungal compounds from peptaibol and furanon compounds by *Trichoderma* sp which can inhibit the growth of pathogenic fungus spores and hyphae. Vermicompost as an organic material has the potential to supply biological agents in the soil.

The presence of organic matter in soil can increase plant resistance to disease, and one example of an organic soil additive is vermicompost. Vermicompost is an organic fertilizer that is produced from the digestive process in a worm's body and disposed of as fermented worm droppings. Vermicompost has many advantages compared to other organic fertilizers because it is rich in essential macro and micronutrients and contains plant growth hormones such as auxin, gibberellins, and cytokines that are required for maximum plant growth (Komang, 2015). Vermicompost also contains antagonistic microorganisms such as *Trichoderma* sp. (Suyono*et al*, 2000 in Susanna *et al*., 2010), thus application of vermicompost into soil can suppress the development of certain plant fungal pathogens such as *Fusarium*. Tomato plants inoculated with a dosage of 100 g per plant of *Fusarium oxysporum sp*. *lycopersici* (Fol) showed *Fusarium* wilt incubation at 7.95 days. Whereas, a much longer incubation period of 19.35 days occurred using a vermicompost treatment of 200 g per plant with two administrations (Susanna *et al*., 2010), indicating the beneficial effect of the vermicompost. At present, there are only a few reports of research on the use of antagonistic microorganisms combined with vermicompost, so this study set out to conduct research by combining vermicompost and *Trichoderma* sp. to investigate their effectiveness in controlling the pathogen *Sclerotium rolfsii*. As previously noted by Susanna *et al*. (2010) the application of vermicompost into soil can directly suppress the development of pathogenic fungi because it contains *Trichoderma* sp. which is antagonistic to Fol. It was explained that increasing the dosage and frequency of vermicompost applications could increase both nutrients, which can encourage healthy plant growth, and the number of antagonistic microorganisms present in the soil so as to reduce the activity of the FoI. Oktarina (2007) states that the application of vermicompost with a ratio of soil and vermicompost as high as 1: 3 per pot one day before planting of the seedlings can reduce the intensity of fungal attack (damping off) caused by *Rizoctoniasolani* in tobacco seedlings by 92.50%. As stated by Oktarina *et al*. (2012), the closer the application time of vermicompost to the nursery, the plant height, number, and width of leaves will increase. It is suspected that the application of vermicompost one week before planting of seedlings is the most appropriate time for the antagonistic fungi contained in the vermicompost to adapt to its environment and protect the tobacco seeds from *Rizoctonia solani* attacks.

The objectives of this study are to: 1) determine the interaction dose of *Trichoderma sp*. and vermicompost which can control the pathogenic attack of *Sclerotium rolfsii* on *Capsicum frutescens* L., variety Cakra Putih, 2) determine the dose of *Trichoderma* sp. which can control the pathogenic attack of *Sclerotium rolfsii* on the chili plant of Cakra Putih varieties and 2) establish the dose of vermicompost that can control the pathogenic attack of *Sclerotium rolfsii*.

Research Method

The study was conducted at the Laboratory and Experimental Sites of the Department of Agronomy, Faculty of Agriculture, Palangka Raya University, using a Factorial Complete Randomized Design consisting of 2 factors, 1) *Trichoderma* sp. dose, namely: T0 = 0 g per box, T1 = 5 g per box, T2 = 10 g per box, T3 = 15 g per box and 2) Vermicompost dose, namely: K0 = 0 g per box, K1 = 100 g per box, K2 = 200 g per box.

Culture medium

Preparation of Potato Sucrose Agar (PSA): Potatoes were peeled and washed and cut into 1 cm size pieces. 200 g was boiled in 1 litre of water until tender. At this point, the potato extract was filtered and poured into a 1-litre glass beaker and reheated in a pan filled with water; 20 g of sugar and 17 g of agar were added to the powder while stirring. After boiling, the extract was removed and 3.64 g of tetracycline was added, and the mixture transferred into 4 x 250 ml erlenmeyers. The media was sterilized by autoclaving at 121 ° C for 30 minutes. After it had cooled, the media was poured into a Petri dish and a sterile test tube (oblique media), until solid.

Culture of Sclerotium rolfsii: *Sclerotium rolfsii* pathogenic isolates were cultured on PSA media in Petri dishes for 7 days and propagated on chopped corn substrate, in stages: chopped maize was washed thoroughly and soaked for 1 hour then steamed until soft. Then 100 g was put into heat-resistant plastic then the plastic ends were folded, stapled and sterilized in an autoclave for 20 minutes at 121°C and 1 atm pressure. After the substrate had cooled, it was inoculated with 5 pieces of *Sclerotium rolfsii* mycelium 5 mm in diameter, and incubated at room temperature for 7 days.

***Culture of Trichoderma* sp.:** *Trichoderma* sp. was obtained from the rhizosphere isolation of banana plants, and cultured on PDA media in a Petri dish on a rice substrate, in stages: the rice was previously washed and soaked for 30 minutes and then steamed until tender. A total of 100 g was put into heat-resistant plastic then the plastic ends were folded then stapled and sterilized in an autoclave for 20 minutes at a temperature of 121°C and a pressure of 1 atm. After the substrate had cooled, it was inoculated with 5 pieces of mycelium *Trichoderma* sp. 5 mm diameter and was incubated at room temperature for 7 days.

Seedbed: Seedlings were grown in the nursery on a substrate that contained a mixture of soil and manure (2:1). The nursery had a protective roof to prevent rainwater and direct sunlight. After 4 weeks of age the seedlings were transferred into a larger box.

Preparation of planting media:The peat soil was air-dried, crushed and sifted with a size of 5 mm and then put into heat-resistant plastic, and sterilized using a drum steamer with a minimum temperature of 100ºC for 6 hours. Subsequently, 3 kg of peat soil was put into the research box.

Inoculation of Sclerotium rolfsii: Up to 10 g of *Sclerotium rolfsii* was inoculated on the planting medium 10 days before planting (3 days before the application of *Trichoderma* sp.), at a depth of 3 cm from the surface of the growing media; it was then covered with thin soil.

Application of Trichoderma* sp. *and Vermicompost: Isolated *Trichoderma* sp. which had been propagated on rice substrate was added at rates of 0, 5, 10 and 15 g per box and Vermicompostat rates of 0, 100, 200 g per box. *Trichoderma* sp. and vermicompostwere added 1 week before planting (3 days after application of *Sclerotium rolfsii*) by sprinkling on *Sclerotium rolfsii* that had been inoculated and then covered with soil.

Chilli Cultivation: Application of dolomite fertilizer at a dose of 26.19 g per plastic box was carried out 1 week before planting. The selected chilli seeds already had 3 leaves, and were planted in each planting medium in a box with as many as 10 chilli seeds per box and with a spacing of 7 cm. An application of 0.48 g of NPK fertilizer per box was done at 2 weeks after planting. Plant cultivation included watering the plants once a day and weeding as required.

Observation

The incubation period for pathogens was observed one day after planting the chilli seeds until the first onset of symptoms. The disease onset was characterized by the occurrence at the base of the plant stem and at the surface of the soil of a regular white mycelium such as feathers or threads and small round sclerotia that were originally white but subsequently turned brown.

The intensity of the disease was determined by the formula:

$$I = \frac{a}{a + b} \times 100\%$$

I = Intensity of disease attack (%)
a = Number of dead plants
b = Number of living plants

Observation of the percentage of dead plants was carried out in the morning, calculated from 1 day after planting (DAP) until the appearance of the attack with an observation interval of 1 week at 1-4 week after planting (WAP).

Sclerotium amount and germination*:Sclerotium* count (of propagules) was done at 4 WAP using the wet sieving technique (Punja *et al.*, 1985) by taking 5 g of planting media on the surface of the media at plastic boxand washing in a filter under running water. The filtrate was dried and the *Sclerotium* propagules were counted manually. *Sclerotium* germination (%) was observed from *sclerotia* propagules thatwere inoculated on PDA media with an incubation mass for 1-3 days. *Sclerotia* germination was calculated using the method of Supriati *et al*. (2007):

$$P = \frac{a}{a+b} \times 100\%$$

P = Percentage of germination (%)
a = Number of germinated sclerotia
b = Number of ungerminated sclerotia

Control Effectiveness, was calculated using the formula according to Sukamto (2003):

$$Ea = \frac{IPk - IPp}{IPk} \times 100\%$$

Ea = Effectiveness of antagonists
GPA = The intensity of the disease in the control
IPp = The intensity of the disease in the treatments

The effectiveness valueswere categorized as Ea> 69% (very good), 50-69% (good), 30-49% (less good) and <30% (not good).

Microbial Population: Antagonistic populations were observed on vermicompost before being applied and after the last observation or 4 WAP on the planting medium. 10 g of the growing media was isolated using a dilution technique (Johnson & Curl, 1972 in Nurhidayat, 2015), put in an erlenmeyer containing 90 ml of sterile water, and stirred until evenly distributed for 30 minutes. A total of 1 ml of this mixture was put into a test tube containing 9 ml of sterile distilled water and then shaken for 1 minute using vortex so that the solution was homogeneous (label as 10^{-1} dilution). Then take 1 ml of the 10^{-1} dilution series and then put into a test tube containing 9 ml of sterile water (labeled as 10^{-2} dilution series) and this method was repeated until the 10^{-4}th dilution series. From the 10^{-4} dilution series, 1 ml was taken from each dilution and inserted into the PSA media. Observation of the growing mushroom culture was carried out for 5 days, and then the*Trichoderma* sp. and fungus in the media were counted (Ginting & Maryono, 2011 in Nurhidayat, 2015). The total microbial population was calculated using the formula according to Damongilala (2009):

Total Microbes (cfu / ml) = number of colonies x dilution factor

Data analysis

Data of attack intensity, number of sclerotia (propagules), plant height and number of leaves were analyzed using analysis of variance (F test) at the levels of α = 5% and α = 1%. Further analysis was carried out with the Honestly Significant Difference (HSD) test at α = 5% level. Data on observations of the incubation period, *Sclerotium* germination and antagonist effectiveness are presented in tabulated form.

Results and Discussion

Incubation Period

The incubation period is the time it takes for the pathogen to infect the plant by entering the plant's body until the initial symptoms appear above ground level. Early symptoms of seedling disease in chillipeppers were found in the base of the stem of the plant, where the base of the stem had been attacked by *Sclerotium rolfsii* following which the plant withers and eventually dies. On the ground surface around the affected plants a white mycelium was also observed which spread like cotton and contained small round sclerotia, which were initially white but subsequently turned brown. Examples of chilli pepper affected by *Sclerotium rolfsii* are presented in Figure 1.

(a) (b)

Figure 1:*Sclerotium rolfsii* attack on chilli peppers, a). Plants look withered, b). The base of the stem shows presence of brown rot

The attack of *Sclerotium rolfsii* on the base of the plants appeared as a rot (Figure 1b) following which the plant stem fell, and the soil around the plant contained mycelium and sclerotia. Agrios (1997) suggests that for disease symptoms to be present there must be a match between the host and the pathogen, and for plants that are sensitive the symptoms of infection from a pathogen can be more quickly seen. The incubation period for *Sclerotium rolfsii* pathogens in the chilli plants is presented in Table 1.

Table 1: Incubation period for pathogenic *Sclerotium rolfsii*

Treatment	Pathogen Incubation Period Range (days after inoculation)
T_0K_0	4 – 5
T_0K_1	10-16
T_0K_2	0 – 18
T_1K_0	0 – 18
T_1K_1	0 – 19
T_1K_2	0
T_2K_0	0
T_2K_1	0 – 18
T_2K_2	0 – 19
T_3K_0	0 – 10
T_3K_1	12 – 18
T_3K_2	0 – 16

Note : Number (0) there were no symptoms of disease attack on chilli pepper until the end of the study. (To) Without *Trichoderma* treatment; (T1) *Trichoderma* 5 g/box; (T2) *Trichoderma* 10 g/box; (T3) *Trichoderma* 15 g/box; (Ko) Without vermicompost; (K1) Vermicompost 100 g/box; (K2) Vermicompost 200 g/box; 0 = does not occur or no symptoms appear after inoculation with the pathogen *Sclerotium rolfsii*

Based on Table 1, the incubation period for pathogens varies between 0-19 DAP, and this variation indicates that the development of antagonists in the planting medium is not the same. The longest incubation period occurs at 19 DAP in the T_1K_1 and T_2K_2 treatments.This is because *Trichoderma* sp. can suppress the growth of *Sclerotium rolfsii*, so the time needed by pathogens to infect plants is longer and the delay in the appearance of symptoms of disease attacks becomes longer. In the treatment of T_2K_0 and T_1K_2 there are no symptoms of *Sclerotium rolfsii* fungus attack because the fungus *Trichoderma* sp. can last for a long period in the soil and can suppress the growth of *Sclerotium rolfsii*. This is related to the higher *Trichoderma* sp. population of 1.67 x 10^4cfu (Table 6) and can inhibit *Sclerotium rolfsii* infection. Thus the incubation period for pathogens does not occur until the end of the observation period. Prabowo *et al*. (2006) in Yudha *et al.* (2016) explained that the delay of the incubation period occurred because of competition between pathogens and antagonists, causing the pathogens to take longer to infect plants because the root system is dominated by antagonists. This condition causes pathogens to compete with antagonists in dominating growth space, on the other hand, antagonists also produce metabolite compounds that can inhibit the growth of pathogens. According to Donzelli *et al*. (2001),*Trichoderma* sp. can produce the enzyme β-1, 3glucanasem that can degrade and hydrolyze the cell wall of mycelium fungal pathogens of plants.During this process the micoparasites remove the contents of the pathogens and the pathogens undergo lysis and then die thereby playing a role in the defence mechanism against pathogens. As an organic soil supplement, vermicompost has the potential to supply beneficial biological agents to the soil. In addition, Octaviani *et al*. (2015) found that Trichoderma sp. produce a large amount of extracellular enzymes β-1,3-glucanase and chitinase during active growth which can dissolve the pathogen cell wall. Trichoderma sp. has the enzyme endokitinase which is a type of chitinase enzyme that has the highest lysis and antifungal activity, such as T. harzianum and T. hamatum capable of producing chitinase and β-1,3-glucanase enzymes which are synergistic in cell wall lysis of S. rolfsii and R. solani so that it can inhibit the growth of these two pathogens.

The T_0K_0 (control) treatment showed the fastest incubation period for pathogens, which was 4 - 5 days after Sclerotium rolfsii pathogen inoculation, while the Trichoderma sp and Vermicompost treatments showed a longer incubation period of the disease so that the symptoms of the disease by Sclerotium rolfsii were slower to occur in cayenne pepper plants. The treatment of Trichoderma sp and Vermicompost can delay the appearance of symptoms of the disease by Sclerotium rolfsii between 10-19 days after inoculation, this is indicated by the treatment T_0K_1, T_0K_2, T_1K_1, T_1K_1, T_2K_1, T_2K_2, T_3K_0, T_3K_1, T_3K_2, no symptoms of the disease appear so that given a value of 0, as well as in the treatment of T_1K_2 and T_2K_0 causes symptoms of the disease did not appear at all and can be said both of these treatments can inhibit the infection of Sclerotium rolfsii in cayenne pepper plants during the study. According to Tjut *et al.,* (2011) the incubation period for *Sclerotium rolfsii* in soybean plants was found to be fastest at a dose of *Trichoderma* 75 g / polybag with an average incubation period of 4.18 days. *Trichoderma* sp. without vermicompost at various doses can inhibit the development of *Sclerotium rolfsii* fungus so that the incubation period that occurs is Vlonger, i.e. between 10-18 DAP. In addition, vermicompost treatment without *Trichoderma* sp. also can inhibit the development of pathogens up to 10-18 DAP, this is because *Trichoderma*

sp. is readily available and has been decomposed with soil that is by applying 7 days before planting. Hardianti (2014) showed that application of *Trichoderma harzianum* 7 days before planting can reduce the percentage of wilting up to 0% in tomato plants. Administration of *T. harzianum* at planting resulted in a percentage of withering of 15.78%. The highest percentage of withering was obtained by giving *T. harzianum* at 7 days after planting, which was 71.74%. The level of effectiveness in preventing *Fusarium* wilt attacks can be seen from the percentage of plant wilting. The most effective treatment in inhibiting the attack of *Fusarium* wilt in tomato plants is the treatment that has the smallest percentage of wilting, namely the treatment of *T. harzianum* at 7 days before planting which has a percentage of wilting 0%.

Intensity of Disease

Based on the results of the analysis of variance, the age of chilli pepper of 1 WAP there was no interaction effect of application *Trichoderma* sp. and vermicompost on the intensity of the attack of seedling on chilli plants, as well as on the single treatment. The interaction effect occurs at ages 2, 3, and 4 WAP. The average intensity of outbreak disease in chilliplants is presented in Table 2.

Table 2: Average Attack Intensity (%) of *Sclerotium rolfsii* Pathogens

Observation time	*Trichoderma* (T)	Vermicompost (K)		
		K_0	K_1	K_2
1 WAP	T_0	10.00	0.00	0.00
	T_1	0.00	0.00	0.00
	T_2	0.00	0.00	0.00
	T_3	0.00	0.00	0.00
HSD 5 %	0.00			
2 WAP	T_0	20.00b	3.33a	0.00a
	T_1	0.00a	0.00a	0.00a
	T_2	0.00a	0.00a	0.00a
	T_3	3.33a	6.67a	6.67a
	HSD 5 %		5.44	
3 WAP	T_0	36.67d	13.33c	3.33ab
	T_1	6.67abc	3.33ab	0.00a
	T_2	0.00a	3.33ab	6.67abc
	T_3	3.33ab	10.00bc	6.67abc
	HSD 5 %		7.68	
4 WAP	T_0	50.00d	13.33c	3.33ab
	T_1	6.67abc	6.67abc	0.00a
	T_2	0.00a	3.33ab	6.67abc
	T_3	3.33ab	10.00bc	6.67abc
	HSD 5 %		7.66	

Note: WAP=week after planting. Figures followed by the same letter at the same age are not significantly different from the 5% HSD test

The attack of seedling disease by *Sclerotium rolfsii* on chillipepper plants begins at age 1-4 WAP. The attack of disease on 1 WAP occurred in the T_0K_0 (control) treatment. On 2 WAPs, the highest intensity of attacks observed at T_0K_0 was significantly different from other treatments. In 3 WAPs, the highest intensity attack in T_0K_0 treatment was significantly different from other treatments, with the highest intensity of other attacks in the T_0K_1 treatment although this was not significantly different from the T_1K_0, T_3K_1, T_2K_2 and T_3K_2 treatments. In 4 WAPs, the highest intensity attack was in the T_0K_0 treatment which was significantly different from the other treatments, and the highest attack intensity was in the T_0K_1 treatment but this was not significantly different from the T_1K_0, T_1K_1, T_3K_1, T_2K_2 and T_3K_2 treatments. In the treatment of T_1K_2 and T_2K_0 symptoms of the disease did not occur even though the dose of *Trichoderma* sp. given was lower but was able to inhibit the development of *Sclerotium rolfsii* so that symptoms of the disease did not appear. This is indicated to be related to higher *Trichoderma* sp. populations in both treatments compared to *Trichoderma* sp. treatments at higher doses (T_3K_1), where *Trichoderma* sp. population is lower so that it is unable to compete and inhibits the development of pathogens causing an increase in the intensity of the attack *Sclerotium rolfsii* from 3-4 WAPs. The low *Trichoderma* sp. population on Vermicompost dose treatment due to competition with bacteria in terms of

space and nutrition, where the bacterial population at Vermicompost is higher (Table 6). Biological control agents in sufficient quantities are generally able to colonize plants quickly and use substrates as well as available nutrients so that they become unavailable to pathogens. Space and nutrition competition is the main mechanism of antagonistic microbes in protecting plant roots from infection or pathogen attack (Manan *et al*., 2018). Some antagonistic microbes can produce enzymes that can hydrolyze chitin, protein, cellulose and hemicellulose (Pal and Gardener, 2006 *in* Manan *et al*., 2018). *Trichoderma* sp from ginger strain is reported to be able to produce chitinase and protease enzymes, the presence of various synergizing compounds can reduce the virulence ability of pathogens (Soesanto *et al*., 2012).

Number of Sclerotial (Sclerotia)

Sclerotium rolfsii has a typical hyphal structure and is not equipped with conidium; when the fungus is in an unfavorable growing environment the fungus will form sclerotia. Sclerotia can persist and will germinate if the growth environment is supportive. New sclerotia are formed after 8–11 days and consist of three layers, namely an inner skin, outer skin, and core skin. The average number of sclerotial (sclerotia) observed at 4 WAP is presented in Table 3.

Table 3: Average Number of Sclerotial (sclerotia) at 4 WAP

Treatment of *Trichoderma* sp.	Vermicompost (K)		
	K_0	K_1	K_2
T_0	24.33d	19.33bcd	9.33a
T_1	8.67a	23.33cd	9.33a
T_2	12.00ab	14.33abc	9.67ab
T_3	10.33ab	15.33abcd	7.66a
HSD 5 %		9.83	

Note : Figures followed by the same letter at the same age are not significantly different from the 5% HSD test

The lowest number of sclerotia occurred in the T_3K_2 treatment while the highest number was in the T_0K_0 treatment but this was not significantly different from T_0K_1, T_1K_1, and T_3K_1.The high number of sclerotia formed was thought to be due to the fungus *Trichoderma* sp. which is used as an antagonist in the treatment but is less able to inhibit the development of pathogenic hyphae, so that pathogenic hyphae that are not inhibited in growth are able to form sclerotia. At high humidities, *S. rolfsii* infection in plants increases, conversely if humidity decreases the extent of disease attack decreases and the mycelium will form sclerotia. In artificial media, sclerotia will germinate in the humidity range of 25-30% (Domsch *et al*., 1980).

The formation of high numbers of sclerotia in the T_0K_0, T_0K_1, T_1K_1, and T_3K_1 treatments, compared with other treatments, indicated that the*Trichoderma* population was likely competing for growing media with other microbes. High bacterial counts indicate that there was competition with *Trichodermasp* that inhibited itsrole as an *S. rolfsii* pathogenic antagonistic agent. According to Supriati *et al*., (2007) in all treatments where *Trichoderma harzianum*was applied with pathogenic cow manure, *S. rolfsii*did not form sclerotia, thus the pathogenic fungi that are parasitized by *T. harzianum* cause degradation and lysis of the pathogenic mycelia and as a consequence mycelia cannot form sclerotia which are a dormant structure from *S. rolfsii*. In the results of this study, sclerotia treated with *Trichoderma* and vermicompost is still growing, indicated by the differences of the types of *Trichoderma* used, where the antagonistic properties of each *Trichoderma are* different, giving different results.

The number of sclerotia formed by *Sclerotium rolfsii* in the treatment of *Trichoderma* sp dose 10 g/box (T2) is relatively high but in this treatment shows the lowest intensity of disease attack. Sclerotium is a means of survival or a hard fungus breeding tool, round, dark in color, able to survive in adverse environmental conditions. *Trichoderma* sp at a dose of 10 g/box which was applied showed good population development (Table 6) so that it could inhibit the development and infection of *Sclerotium rolfsii* in chili pepper plants. The inhibition of antagonists (*Trichoderma* sp) causes an unfavorable condition for the development of *Sclerotium rolfsii* so that sclerotia is formed as a means of self defense. Juariyah *et al*. (2018) reported that each application of 10 g/kg of inoculum of some *Trichoderma* sp lines was able to inhibit the development of *Fusarium* spp. (Papua strain) in oil palm seedlings with a resistance level of 59.74-90.91%. *Trichoderma* sp application before planting will give the opportunity to *Trichoderma* sp to colonize the roots and rhizosphere first, thus when the pathogen is inoculated in the planting medium the pathogen cannot develop properly and cannot infect the plant so that the plant is protected from pathogens especially soil infectious pathogens (Harman *et al*., 2004 *in* Juariyah *et al*., 2018).

Percentage germination of sclerotia

Sclerotia germination is observed to be dispersive (i.e. there are hyphae emanating from all angles of the

sclerotia) with fine branched threads shaped like white compact and solid cotton, and with the smallest diameter size of 0.61 cm seen on the second day and the largest size of 1.71 cm seen on the seventh day (Magenda*et al.,* 2011). The percentage of sclerotia germination can be seen in Table 4.

Table 4: Percentage of sclerotia germination 1 day after incubation

Treatment	Germination (%)
T_0K_0	100
T_0K_1	97.33
T_0K_2	100
T_1K_0	100
T_1K_1	97.22
T_1K_2	100
T_2K_0	100
T_2K_1	47.56
T_2K_2	100
T_3K_0	100
T_3K_1	93.33
T_3K_2	100

The lowest germination at 1 day after incubation was in the T_2K_1 treatment while the highest germination was in the T_0K_0, T_0K_2, T_1K_0, T_1K_2, T_2K_0, T_2K_2, T_3K_0, T_3K_2 treatments. Indications for sclerotia that have formed are difficult to penetrate the antagonist *Trichoderma* sp. so it is difficult to penetrate the pathogen *S. rolfsii*. Like other fungi that have hyphae, hypha of S. rolfsii does not form spores but sclerotia. In artificial media, sclerotia only form after 8–11 days. Sclerotia consist of three layers, namely inner skin, outer skin, and terrace skin. In the inner skin, there are 6-8 cell layers, outer skin 4-6 cell layers, while the core skin consists of hyphae threads that are hyaline and do not experience thickening of the cell wall (Chet *et al*. 1969 in Sumartini, 2012).

Control Effectiveness

Application of *Trichoderma* sp. and vermicompost both singly and in combination can control the pathogenic *Sclerotium rolfsii*. This ability is reflected in the effectiveness value of *Trichoderma* sp. and vermicompost (Figure 2).

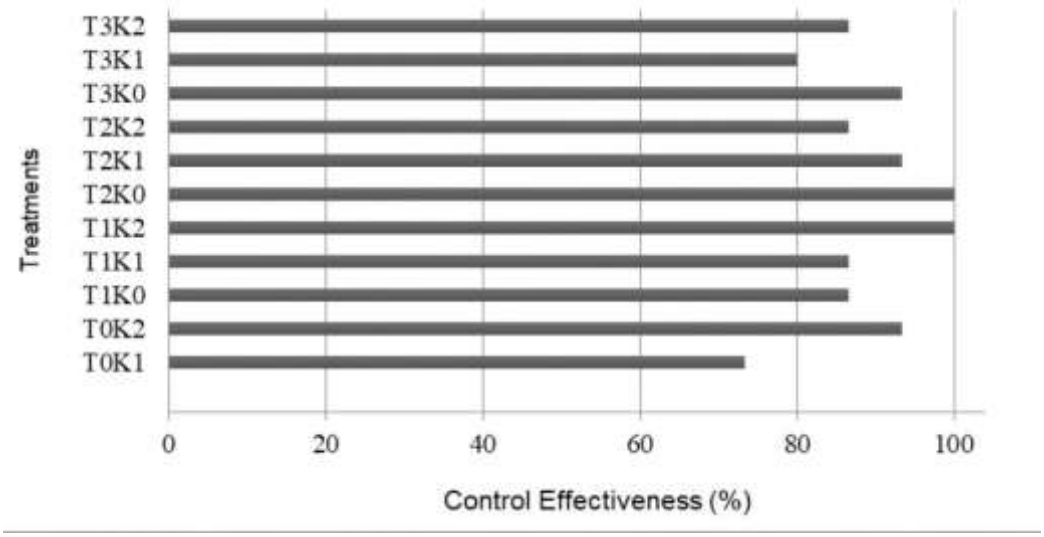

Figure 2: Effectiveness of *Trichoderma* sp. and vermicompost in suppressing nursery desease at 4 WA

The effectiveness of control using *Trichoderma* sp. combined with vermicompost was between 73.3 - 100%, is categorized very well because the value of effectiveness in controlling deseases> 69%. According to Sukamto (2003) the effectiveness of antagonists > 69% is categorized very good. The highest control effectiveness was found in the T_2K_0 and T_1K_2 treatments with a value of 100%. In the treatment of T_2K_0 with a

combined dose of vermicompost 0 g and *Trichoderma* sp. 10 g, the growth of pathogens occurred because *Trichoderma* sp. suppressed the population of pathogenic fungi. Interactions begin by the *Trichoderma* hyphae wrapping around the pathogenic fungus which forms a hook-like structure called a haustorium; the pathogenic parasite then releases an enzyme that can degrade and hydrolyze the cell wall of the pathogenic fungus during the process of paracitating and removing the contents of the pathogen so that the pathogen undergoes lysis and then dies releasing enzymes that can degrade and hydrolyze the cell wall of mycelium (Papavizas, 1985 *in* Tjut *et al*., 2011).

The existence of antagonistic microorganisms in the soil is very desirable for a long period of time. For this reason, it is necessary to provide a food supply that is able to maintain the survival of these biological agents. According to Baker & Cook (1974) in Sunarti *et al*. (2015) organic matter which is applied to the soil is a nutrient source for antagonistic microorganisms; it can promote increased activity, stimulate dormant propagule dormancy and produce fungistatic effects for soil infectious pathogens. The ability of vermicompost to increase the activity of soil microorganisms occurs not only during the application of the vermicompost, but can also last for a long time afterwards. According to Mulat (2003) microorganisms can continue to develop almost one year after being given vermicompost treatment.

Microbial Populations

Vermicompost contains complete nutrients, micronutrients and macronutrients as well as various antagonistic microorganisms and growth-stimulating hormones that are useful for plant growth, such as gibberellin, cytokines and auxins (Mulat, 2003). Observation of microbial populations before planting on vermicompost is presented in Table 5, and the average microbial population after planting with chilli plants can be seen in Table 6.

Table 5: Observation of microbial populations before planting at vermicompost

Treatment	Microbe	Total Population (cfu/10 g of soil) ($10^{4)}$
Vermicompost media	*Trichoderma* sp.	1
	Bacteria	10
	Other Fungi	0

Table 6: Average microbial population after planting on chili

Treatment	Microbe	Total Population (cfu/10 g soil) (10^4)
T_0K_0	*Trichoderma* sp.	0.33
	Bacteria	13.00
	Other Fungi	2.33
T_0K_1	*Trichoderma* sp.	1.33
	Bacteria	18.33
	Other Fungi	4.33
T_0K_2	*Trichoderma* sp.	0.33
	Bacteria	14.33
	Other Fungi	3.00
T_1K_0	*Trichoderma* sp.	1.33
	Bacteria	11.00
	Other Fungi	1.67
T_1K_1	*Trichoderma* sp.	0.67
	Bacteria	23.33
	Other Fungi	3.67
T_1K_2	*Trichoderma* sp.	1.67
	Bacteria	12.67
	Other Fungi	4.00
T_2K_0	*Trichoderma* sp.	1.67
	Bacteria	15.67
	Other Fungi	4.33
T_2K_1	*Trichoderma* sp.	1.33
	Bacteria	17.67
	Other Fungi	3.67
T_2K_2	*Trichoderma* sp.	0.67
	Bacteria	17.67
	Other Fungi	3.33
T_3K_0	*Trichoderma* sp.	1.33
	Bacteria	15.00
	Other Fungi	3.00
T_3K_1	*Trichoderma* sp.	0.67
	Bacteria	12.33
	Other Fungi	3.00
T_3K_2	*Trichoderma* sp.	2.00
	Bacteria	17.67
	Other Fungi	3.00

The microbial population increases after planting compared to the microbial population found in the vermicompostbefore planting. Before planting there was a total population of *Trichoderma* sp. 1 cfu, 10 cfu bacteria in the 10^{-4} dilution series. According to Oktarina et *al*. (2012), by diluting vermicompost into 10 ml of aquadest up to 10^{-6} which are then implanted on PDA media, it is known that in vermicompost used there are antagonistic fungus *Trichoderma* sp. The activity of antagonistic microorganisms that suppress the intensity of an *Rhizoctonia solani* attack is in line with previous studies that show that *Trichoderma* sp. are able to inhibit the development of lodging diseases (Murdan and Thoyibah, 1997). Based on the results of Komang's research (2015) the dose of vermicompost fertilizer has a very significant effect on the total population of microorganisms. This is because an increase in the concentration of vermicompost in the soil will stimulate the activity and propagation of microorganisms. Rao (1994) reports that as more organic fertilizer is put into the soil, the development of soil microorganisms will increase. This is because the organic material contained in organic fertilizer is used as a source of life and according to Hakim *et al*. (1986) by applying organic material into the soil will improve the life of microorganisms in the soil.

The presence of microbes in the soil is strongly influenced by the level of acidity (pH). Fungi are found in acidic environments, whereas bacteria can live across a wider pH range (Sylvia, 2005). Bacteria are the most abundant group of microbes in rhizosphere, their numbers ranging from 106 - 109 organisms per gram of rhizosphere soil(Sylvia, 2005).

Conclusion

Based on the results of the study, we can make the following conclusions:

There is an effect of the application of *Trichoderma* sp. and vermicompost in controlling seedling disease by *Sclerotium rolfsii* on 2, 3 and 4 WAP. The T_1K_2 and T_2K_0

treatments are the best treatments within the incubation period parameters with no disease symptoms occurring in the treatment. The disease control effectiveness was in the 'very good' category with a range of control effectiveness values from 73.3 to 100%.The highest number of sclerotia occurred in the control treatment (T_0K_0), and the lowest percentage of sclerotia germination in the T_2K_1 treatment with the percentage of germination at 47.6%.

The application of vermicompost significantly affected the height of the chilli plants aged 2, 3 and 4 WAP, but there was no effect of interaction of *Trichoderma* sp. and vermicompost, nor of a single treatment of *Trichoderma* sp. against plant height. Supplying *Trichoderma* sp. at doses of 5, 10 and 15 g / box significantly affected the number of plant leaves aged 2 WAP, but there was no effect on the number of leaves of the interaction of *Trichoderma* sp. and vermicompost and the vermicompost single treatment.

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