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Potential of Dosage of Bokashi from Agricultural Waste and Bio-decomposer *Trichoderma asperellum* on Growth of Three Varieties of Soybean, and Disease Incidence of Stem Rot Caused by *Sclerotium rolfsii*

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Abstract: *This research aims to determine the effect of interaction and the main effect of soybean varieties with a dose of bokashi from agricultural waste and Trichoderma asperellum, besides, to determine the best dosage of bokashi on soybean growth and disease incidence of stem rot caused by Sclerotium rolfsii. The research design uses a factorial randomized block design, following the first factor is soybean varieties, namely V1 (Anjasmoro), V2 (Argomuliyo), and V3 (Dena). Furthermore, the second factor is the bokashi dosage, namely T0 (dose of 0 tons/ha), T1 (5 tons/ha), T2 (10 tons/ha) and T3 (15 tons/ha). All data obtained were analyzed using analysis of variance. If the effect is significant, then continue with Duncan Multiple Range Test (DMRT) 95% confidence level. The results showed that the best variety of growth is Anjasmoro, while a variety of Dena is the best for reducing stem rot disease incidence and more resistance from other varieties. Bokashi dose of 15 tons/ha is the most effective in increasing soybean growth and bokashi dose of 10 tons/ha is effective in suppressing stem rot disease incidence caused by Sclerotium rolfsii.*

Keywords: *Bokashi dosage, soybean varieties, Sclerotium rolfsii, Trichoderma asperellum*

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I. Introduction

Soybean (*Glicyne max* L.) is the third main food commodity after rice and corn. Soybean is very important for improving people's nutrition because the price is relatively cheap and affordable by all levels of society when compared with other protein sources such as meat, fish, and milk (Septiatin, 2012).

Soybean production in Indonesia in 2015 was 963.183 tons and went down in 2016 and 2017 respectively of 859.653 tons and 538.728 tons, and then increased to 982.598 in 2018. Meanwhile, soybean production in Southeast Sulawesi in 2015 was 12.799 tons and increased in 2016 of 16.176 tons, however, it went down again in 2017 of 4.055 tons and then got increase again in 2018 of 8.007 tons. Increase of soybean production not offset with soybean needs in Indonesia and Southeast Sulawesi, so that those productions have not been able to fill people's needs (Central Bureau of Statistics, 2018).

Some problems in soybean cultivation are the use of superior soybean varieties which is still not optimal and the problem of plant-disturbing organisms such as pests and plant diseases. The important plant-disturbing organisms that attack soybean is a fungus *Sclerotium rolfsii* that causes stem rot, wilt, and damping-off of plant, and it is a soil-borne pathogen and be able to live in the soil for a long time with a form of sclerotia (Nengtias et al., 2012). The disease is often found on dry land, rainfed and tidal land with disease intensity reaching 55% and causing yield loss of 40%, if environmental conditions are favorable for disease development, then the losses can reach 100% (Sumartini, 2011). Generally, *S. rolfsii* lives in the tropics, its distribution is almost in all regions of Indonesia include Southeast Sulawesi. The most farmer still relies on chemical pesticides continuously for control the disease without considering the negative impacts in the soil and plant (Nengtias et al., 2012).

The utilizing of bokashi with *Trichoderma* sp. can be used to reduce the use of chemical pesticides. Bokashi has two functions that can increase plant growth and able to suppress the growth of plant pathogens. *Trichoderma* sp. can break down organic materials, produce growth hormones such as cytokinins and auxins, have antagonistic abilities against plant pathogens. Also, the use of superior varieties can be done to increase soybean crop production, to be able to achieve high yields is largely determined by genetic potential. There are several soybean varieties are known in Southeast Sulawesi namely Anjasmoro, Agromulio, and Dena.

This research use biofertilizer based on *Trichoderma asperellum* as a bio-decomposer bokashi from agricultural waste to increase plant growth and control plant disease caused by *Sclerotium rolfsii*. Besides, to determine the effect of interaction and the main effect of soybean varieties with a dose of bokashi from agricultural waste and *Trichoderma asperellum*, and to determine the best dosage of bokashi on soybean growth and disease incidence of stem rot caused by *Sclerotium rolfsii*.

II. Materials and Methods

This research process started from land preparation, preparation of *Trichoderma asperellum*, making bokashi from agricultural waste plus *T. asperellum*, liming, application of bokashi plus *T. asperellum*, planting, application of inorganic fertilizer, plant maintenance.

The research design uses a factorial randomized block design, following the first factor is soybean varieties, consisting of three levels, namely V1 (Anjasmoro), V2 (Argomuliyo), and V3 (Dena). Furthermore, the second factor is the bokashi dosage, consisting of four levels, namely T0 (dose of 0 tons/ha), T1 (dose of 5 tons/ha), T2 (dose of 10 tons/ha) and T3 (dose of 15 tons/ha). So, there are 12 treatment combinations.

The research variables were observed namely growth variables and disease variables. Growth variables such as plant height and the number of leaves, while disease variables such as the initial appearance of the disease, symptoms of the disease, and disease incidence. Observation of disease incidence is calculated based on symptoms with the following formula:

$$KP = \frac{n}{N} \text{ x } 100\ \%$$

where: KP is disease incidence,
n is number of diseased plants,
N is the number of plants observed.

Besides, to the value of disease incidence, AUDPC (Area Under Disease Progress Curve) values are also calculated to see the overall disease development. AUDPC values are calculated based on the Van der Plank formula (1963) as follows:

$$\text{AUDPC} = \sum_{I}^{n=1} \left(\frac{\text{yi} + \text{yi}_{+1}}{2}\right) \text{x } \text{t}_{\text{i}+1} - \text{ti})$$

where : y_{i+1} is observation data i+1
y_i is observation data i
t_{i+1} is time of observation i
t_i is time of observation 1

The effectiveness of bokashi from agricultural waste and decomposer *Trichoderma asperellum* on the disease incidence of stem rot disease in soybean plants is calculated to use formula as follows:

$$EP = \frac{DIK - DIP}{DIK} x 100\ \%$$

where: EP is the effectiveness of disease suppression (%)
KPK is AUDPC on control
KPP is AUDPC on Treatment

All data obtained were analyzed using analysis of variance. If the effect is significantly real, then continue with Duncan Multiple Range Test (DMRT) 95% confidence level.

III. Results and Discussion

Results

Plant Height

The main effect of several soybean varieties and bokashi dosages on plant height two, four, six, and eight weeks after planting is presented in Table 1 and Table 2.

Table 1. Independent effects of several soybean varieties on plant height.

Type of varieties	Average of plant height (weeks after planting)							
	2		4		6		8	
Anjasmoro (V1)	11.43	a	20.55	a	38.30	a	45.98	a
Agromulyo (V2)	9.69	b	18.23	b	30.03	c	39.93	b

Dena (V3)	9.75	b	18.53	b	31.83	b	36.02	c
DMRT 0,05α	2=0.55 3=0.58		2=0.72 3=0.76		2=1.59 3=1.67		2=1.74 3=1.83	

Note: The numbers followed by the same letters in the same column are not significantly different in the DMRT 0.05α.

The DMRT results showed that the highest value is in the V1 treatment (Anjasmoro variety) and significantly different from treatments V2 (Argomulyo) and D3 (Dena).

Table 2. The main effect of the dose of bokashi from agricultural waste and *Trichoderma asperellum* on plant height.

Dosages of bokashi	Average of plant height (weeks after planting)							
	2		4		6		8	
0 Ton/Ha (T0)	8.92	c	17.09	c	26.62	d	32.13	c
5 Ton/Ha (T1)	10.13	b	18.36	b	32.60	c	39.98	b
10 Ton/Ha (T2)	10.69	ab	19.18	b	35.31	b	41.16	b
15 Ton/Ha (T3)	11.42	a	21.80	a	39.02	a	49.31	a
DMRT 0,05α	2=0.74 3=0.77 4=0.8		2=0.97 3=1.01 4=1.04		2=2.12 3=2.23 4=2.29		2=2.32 3=2.44 4=2.52	

Note: The numbers followed by the same letters in the same column are not significantly different in the DMRT 0.05α.

The DMRT results showed the highest value obtained from the T3 treatment (dose of 15 tons/ha), while the lowest plant height was in the T0 treatment (dose of 0 tons/ha).

Number of Leaves

The main effect of the dose of bokashi on the number of leaves four, six, and eight weeks after planting is presented in Table 3.

Table 3. The main effect of the dose of bokashi from agricultural waste and *Trichoderma asperellum* on the number of leaves.

Dosages of bokashi	Average of number of leaves (weeks after planting)		
	4	6	8
0 Ton/ha (T0)	5.24 c	8.87 d	14.36 c
5 Ton/ha (T1)	5.80 b	11.27 c	19.02 b
10 Ton/ha (T2)	5.82 b	12.58 b	20.78 a
15 Ton/ha (T3)	6.38 a	13.91 a	21.69 a
DMRT 0,05α	2=0.29 3=0.31 4=0.32	2=1.07 3=1.13 4=1.16	2=1.19 3=1.25 4=1.29

Note: The numbers followed by the same letters in the same column are not significantly different in the DMRT 0.05α.

The results of the DMRT showed the highest number of leaves is in the T3 treatment (dose of 15 tons/ha) and is not significantly different from the T2 treatment (dose of 10 tons/ha) at eight weeks after plants. The lowest number of leaves was found in the T0 treatment (dose of 0 tons/ha) and significantly different from other treatments.

Disease Variable

1. Early emergence of symptoms of the disease

Early emergence of stem rot disease in the field begins with an infection in the roots or stems bordering the soil ground surface. The infection causes the transportation of nutrients and water is blocked and plant wilt in the end. The average initial appearance of disease symptoms in each treatment can be seen in Table 4.

Table 4. The average of the initial appearance of stem rot disease symptoms caused by *Sclerotium rolfsii* at each treatment.

Treatments	The average of the initial appearance of stem rot disease symptoms (days after planting)
V1TO (Anjasmoro + without dosage of bokashi)	46.67
V1T1 (Anjasmoro + dose of 5 ton/ha)	56.00
V1T2 (Anjasmoro + dose of 10 ton/ha)	-
V1T3 (Anjasmoro + dose of 15 ton/ha)	63.00
V2T0 (Agromulyo + without dosage of bokashi)	56.00
V2T1 (Agromulyo + dose of 5 ton/ha)	43.00
V2T2 (Agromulyo + dose of 10 ton/ha)	63.00
V2T3 (Agromulyo + dose of 15 ton/ha)	-
V3T0 (Dena + without dosage of bokashi)	42.00
V3T1 (Dena + dose of 5 ton/ha)	63.00
V3T2 (Dena + dose of 10 ton/ha)	-
V3T3 (Dena + dose of 15 ton/ha)	-

Note: (-) = no symptoms

Table 4. shows that the average of the fastest emergence of disease is in the V3T0 treatment of 42.00 (days) and followed by the V2T1 treatment of 43.00 (days), while in the treatments V1T2, V2T3, V3T2, and V3T3 showed no symptoms of the disease.

2. The Symptom

Symptoms of *Sclerotium rolfsii* disease are brownish spots around the base of the stem and then the leaves turn yellow withered on the branches and eventually die. The pathogen then spreads to all parts of the plant and causes decay. There are white mycelium or sclerotia on the soil surface around the infected plants. In laboratory observations, it was found that *S. rolfsii* mycelium is white like a feather. Symptoms of stem rot disease and hyphal form of *S. rolfsii* in the field can be seen in Figure 1.

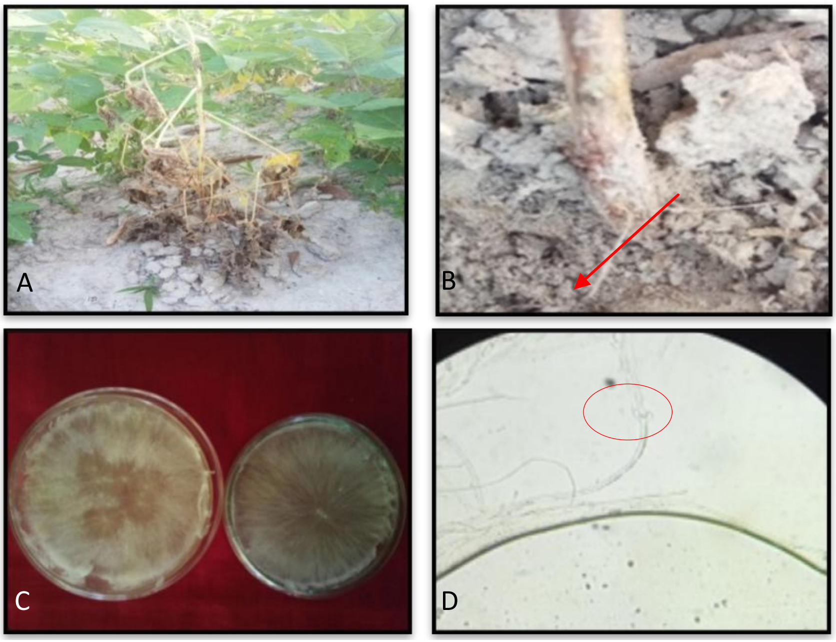

Figure 1. Symptoms of stem rot disease in soybean plants (a): symptoms in the field, (b): *S. rolfsii* sclerotia at the base of the stem, (c): *S.rolfsii* growing on PDA medium, (d): clam connections of *S. rolfsii* mycelium.

Disease incidence

The main effect of some varieties on the disease incidence of stem rot in the observation of nine weeks after planting (WAP) is presented in Table 5. The main effect of the dosage of bokashi on the observation of eight and nine weeks after planting (WAP) is presented in Table 6.

Table 5. The main effect of soybean varieties on the disease incidence of stem rot caused by *S. rolfsii* on soybean (after transformation to $\sqrt{(x + 0,5)}$).

Type of varieties	Average of disease incidence (%)
	9 WAP
Anjasmoro (V1)	3.98 a
Agromulyo (V2)	1.93 b
Dena (V3)	0.80 c
DMRT 0,05α	2=0.26
	3=0.27

Note: The numbers followed by the same letters in the same column are not significantly different in the DMRT 0.05α.

The DMRT results of the main effect of three soybean varieties on the disease incidence (Table 5.) showed that the lowest value in the treatment of V3 (Dena variety) that were significantly different from V2 and V1.

Table 6. The main effect of the dose of the bokashi from agricultural waste and *Trichoderma asperellum* on the disease incidence of stem rot on soybean (after transformation to $\sqrt{(x + 0,5)}$).

Dosages of bokashi	Average of disease incidence (%)	
	8 WAP	9 WAP
0 Ton/Ha (T0)	3.58 a	5.16 a
5 Ton/Ha (T1)	1.12 b	2.77 b
10 Ton/Ha (T2)	0.00 c	0.62 c
15 Ton/Ha (T3)	0.00 c	0.40 c
DMRT 0,05α	2=0.32	2=0.34
	3=0.33	3=0.36
	4=0.34	4=0.37

Note: The numbers followed by the same letters in the same column are not significantly different in the DMRT 0.05α.

The DMRT results showed that the best dosage of bokashi is in treatments of T3 (15 ton/ha) dan T2 (10 ton/ha).

AUDPC Incidence of Trunk Base Rot Disease

The results of the stem rot disease development of soybean at each treatment is done by calculating the AUDPC value. Results of the average AUDPC value of the stem rot disease incidence of stem rot disease are presented in Table 7.

Table 7. The main effect of the dose of bokashi from agricultural waste and *Trichoderma asperellum* on AUDPC stem rot disease incidence of soybean.

Dosages of bokashi	Average AUDPC of disease incidence
	AUDPC
0 Ton/Ha (T0)	61.65 a
5 Ton/Ha (T1)	38.82 b
10 Ton/Ha (T2)	21.06 c
15 Ton/Ha (T3)	20.32 c
DMRT 0,05α	2=10.16
	3=10.68

4=10.99

Note: The numbers followed by the same letters in the same column are not significantly different in the DMRT 0.05α.

The DMRT results showed that the lowest stem rot disease incidence is in T3 treatment (dose of 15 ton/ha) and it is not significantly different from the T2 treatment (dose of 10 tons/ha). The results of the average AUDPC stem rot disease incidence of three varieties are presented in Figure 2.

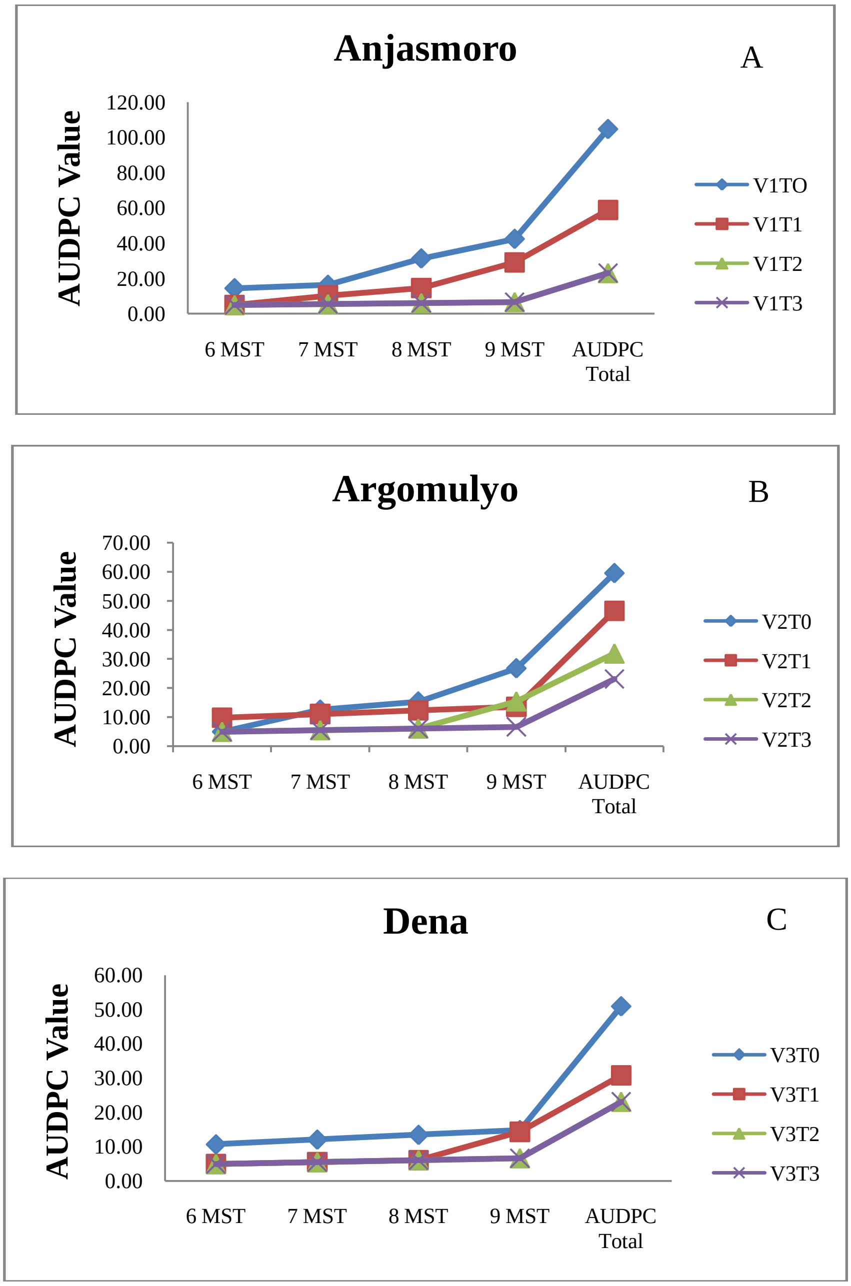

Figure 2. AUDPC value of disease development of three varieties of soybean, where A: Anjasmoro variety, B: Argomulyo variety, and C: Dena variety.

Effectiveness of disease suppression

The results of observations of the effectiveness of suppression of stem rot disease caused by *S. rolfsii* which were treated by several doses of bokashi from agricultural waste and *T. asperellum* of three varieties of soybean are presented in Table 8.

Table 8. Effectiveness of suppression of stem rot disease incidence in several soybean varieties.

Treatments	Total of AUDPC	Effectiveness of disease suppression
V1TO (Anjasmoro + without dosage of bokashi)	104.63	0.00%
V1T1 (Anjasmoro + dose of 5 ton/ha)	58.82	43.78%
V1T2 (Anjasmoro + dose of 10 ton/ha)	23.06	77.96%
V1T3 (Anjasmoro + dose of 15 ton/ha)	23.06	77.96%
V2T0 (Agromulyo + without dosage of bokashi)	59.47	0.00%
V2T1 (Agromulyo + dose of 5 ton/ha)	46.48	21.84%
V2T2 (Agromulyo + dose of 10 ton/ha)	31.83	46.48%
V2T3 (Agromulyo + dose of 15 ton/ha)	23.06	61.22%
V3T0 (Dena + without dosage of bokashi)	50.91	0.00%
V3T1 (Dena + dose of 5 ton/ha)	30.76	39.58%
V3T2 (Dena + dose of 10 ton/ha)	23.06	54.70%
V3T3 (Dena + dose of 15 ton/ha)	23.06	54.70%

The results of the average effectiveness of stem rot disease suppression showed V1T2, V1T3, V2T2, V2T3, V3T2, and V3T3 are the best treatments that able to suppress stem rot disease caused by *S. rotfsii*.

IV. Discussion

The use of local biological natural resources, organic materials, biological fertilizers, resistant plant varieties and minimize the use of chemical materials are an effort to be done in the success of plant cultivation to get high production and be able to assist in the development of soybean plants in Indonesia, especially in Southeast Sulawesi to fill the national consumption needs that are increasing every year.

The results of observations on plant height at two to eight weeks after planting on the effect of varieties (Table 1.) showed that the variety that gave the best response to plant height was Anjasmoro variety compared to other varieties, this happened due to genetic differences in various types of varieties and factors of favorable environment, these genetic differences cause differences in the appearance of plant phenotypes with special characteristics and natures. Irwan (2006) states that varieties play a high role largely determined by the potential yield of superior varieties planted. The potential yield of seeds in the field is still affected by the interaction between genetic factors of the variety and the management of growing environmental conditions. Furthermore, if the management of the growing environment is not carried out properly, the potential for high yield of seeds from these superior varieties cannot be achieved.

The treatments of dosages of bokashi at two, four, six, and eight weeks after planting (Table 2.) showed that the main effect of doses that be able to increase plant height is most effective at treatment with a dose of 15 tons/ha. Giving a dose of 15 tons/ha also provides good performance on the number of leaves at four, six and eight weeks after planting (Table 3.), it is assumed that the higher the amount of organic material applied, then the results are increasing. Rooting that has been applied by *Trichoderma* sp. causing optimum absorption of nutrients so that plants be able to grow well (Ismail et al, 2011). Vessey (2003) added that the application of organic fertilizers containing living microorganisms, which when applied to seeds, plant surfaces, or soil can stimulate plant growth.

Observation of the disease incidence of *Sclerotium rolfsii* at nine weeks after planting (Table 5.) showed that the lowest disease incidence is in the Dena variety. Dena variety is most resistant from other varieties due to it has genetic and vertical resistance, incompatible occurrence between the host and pathogens, so that there is no infection and disease. Genetic resistance is more meaningful if it can provide good and comprehensive protection to plants (Abadi, 2003).

Observation of disease occurrence at eight and nine weeks after planting (Table 6.) and AUDPC disease progression showed the lowest of disease incidence and disease progression namely in treatment bokashi with dosages of 15 tons/ha and 10 tons/ha. The low of disease incidence due to dosages of 10 tons/ha to 15 tons/ha of bokashi is balance with *T. asperellum*, so that *T. asperellum* be able to grow well and helping increase soybean plant health. The right dosage of bokashi can effect increasing the activity of microorganisms especially *T. asperellum* which colonize the soybean plant rhizosphere so that the system can induce plant resistance to pathogenic infections that cause plant disease. *Trichorderma* sp. are microparasites that attack many types of plant pathogens and is beneficial for plants (Gusnawaty et al, 2014), moreover, *Trichoderma* sp. can break down nutrients that are bound in the soil, produce glycotoxic and viridian antibiotics that be able to used to protect plant seeds from disease and release enzymes of glucanase and chitinase that can breakdown cell wall of pathogens (Ismail et al, 2011).

The emergence of stem rot disease caused by *S. rolfsi* starts from the remnants of infected plants in the field, and form sclerotia to survive in the soil. This pathogen is known to have a broad range of hosts included in the group of legumes such as soybeans. Figure 2 (A, B and C) showed that the rate of disease progression from the six to nine weeks after planting in Anjasmoro and Dena varieties each week has increased in varieties that were given by dosages of 0 tons/ha and 5 tons/ha of bokashi, while the treatment with the development of stem rot disease incidence is relatively stable with the treatment of dosages of 10 tons/ha and 15 tons/ha of bokashi.

The growth of *Trichoderma* sp. in the soil after the application is very important to support its ability to suppress disease. *Trichoderma* sp. is one type of antagonistic fungus that is easy to develop and adapt to soil media. Besides, this fungus has high persistence that can inhibit the development of pathogenic fungi. Recent studies conducted by Bailey et al. (2009) showed that *T. asperellum* can suppress the development of *Phytoptora palmivora* on cocoa plants, he stated that *Trichoderma* sp. can penetrate the cocoa plant tissue and association with it.

The average of *Sclerotium rolfsii* disease treated with doses of bokashi on three types of soybean plant varieties (Table 8.) showed that the three varieties of soybean that were given by doses of 10 tons/ha and 15 tons/ha bokashi are the best treatments compared to the treatment without a dose of bokashi respectively 77.96%, 61.22%, and 54.70%, this indicates that given of different doses of bokashi to each variety is very effective in controlling *Sclerotium rolfsii*, because the combination of these treatments can increase the survival and activity of microorganisms in the soil. The provision of *T. asperellum* which is applied with the carrier media such as organic material from agricultural waste on beds affects the *S. rolfsii* disease incidence in the field. Djafaruddin (2000) states that the culture of *Trichoderma* sp. can act as a bio-decomposer that decomposes the organic waste into quality compost and a bio-fungicide that be able to control pathogenic organisms that cause disease. The number of antagonistic fungus mycelia is very dependent on the number of inoculums that are introduced and balanced organic materials in the soil. The more of antagonistic fungus mycelia *Trichoderma* sp. are produced in the soil would be able to cause the infection of *S.rolfsii* fungi in soybeans to get smaller because *S. rolfsii* is less able to develop due to competition in space and nutrition with *Trichoderma* sp. due to it producing antibiosis compounds sich as gliotoxins, glioviridin, and viridiol, or mycoparasites. *Trichoderma* sp. which are microparasites 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 parasitize the fungus pathogen.

V. Conclusion

There is no effect of interaction between soybean varieties and the dose of bokashi from agricultural waste and bio-decomposer *Trichodema asperellum* on the plant growth and stem rot disease incidence of soybean, while there are the main effect of all varieties and dose of bokashi of soybean height at two, four, six and eight weeks after planting, and number of leaves at four, six and eight weeks after planting, disease incidence at eight and nine weeks after planting. Anjasmoro is the best main effect of soybean variety on plant height, while Dena is the lowest disease incidence of soybean variety. The best dosage of bokashi for soybean plant growth is 15 tons/ha and the effectiveness and efficient dose for stem rot disease is 10 tons/ha.

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