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THE EFFECTIVENESS OF BOKASHI BIO-DECOMPOSER AND DOSAGE OF INORGANIC FERTILIZER ON GROWTH AND DISEASE INCIDENCE OF VIRUS SYMPTOM OF SOYBEAN

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ABSTRACT

This study aims to examine the effectiveness of a combination of bokashi decomposers and dosages of inorganic fertilizer on growth and disease incidence caused by viruses in soybean. This research uses a randomized block design consisting of 2 factors, namely B: bokashi decomposers and A: dosages of inorganic fertilizer. The first factor consists of several levels of treatments, that is B0: without decomposers, B1: Microorganisms-4 (EM4), B2: *Trichoderma asperellum*, B3: Microorganisms-4 (EM4) + *Trichoderma asperellum*, while the second factor is A0: without inorganic fertilizer, A1: 25% of the recommended dose of inorganic fertilizer, A2: 50% of the recommended dose of inorganic fertilizer, A3: 75% of the recommended dose of inorganic Fertilizer and A4: 100% of the recommended dose of inorganic fertilizer. Each treatment is combined to produce 20 treatment combinations. Observation variables which are observed that is growth variables consisting of plant height and the number of leaves, while disease observation variables consisting of the first emergence of plant disease symptoms, types of disease based on symptoms, plant disease incidence, Area Under Disease Progress Curve (AUDPC) and the effectiveness of disease suppression (based on AUDPC). The results of observations showed a significant effect, so that was continued with the Duncan Multiple Range Test (DMRT) at the significance level $\alpha = 0.05$. The results showed that the main treatment of *Trichoderma asperellum* (B2) and 100% of the recommended dose of inorganic fertilizer (A4) were significantly able to increase plant growth and reduce the incidence of the disease that suspect was caused by viruses in soybean plants.

Keywords: *Decomposers, soybean, Trichoderma asperellum.*

I. INTRODUCTION

The soybean plant is the third-most important food commodities after rice and maize. In 2016, soybean production in Indonesia amounted to 859.653 tons and went down in 2017 amounted to 538.728 tons. Likewise, in 2016, soybean production in Southeast Sulawesi reached 16.136 tons and fell in 2017 to only 4.055 tons. It happened due to soybean needs in Indonesia are not offset by domestic soybean production and cause low soybean productivity (Central Bureau of Statistics, 2018).

One of the causes of low production is the presence of plant diseases. Especially diseases caused by viruses such as Soybean Mosaic Virus (SMV), Cowpea mild mottle virus (CPMMV), and Cucumber mosaic virus (CMV). They can cause the biggest losses to soybean plants (Taufik et al., 2015).

Currently, disease management in agriculture more focused on eco-friendly management by using microorganisms such as *Trichoderma* sp. *Trichoderma* sp. is a microorganism antagonistic which is antagonistic to some plant pathogens, in addition, has a function as a decomposer, increase plant growth, and can be used as organic fertilizer or biofertilizer.

The results of previous studies show that the indigenous fungi of Southeast Sulawesi namely *Trichoderma asperellum* are able to decompose organic matter quickly (Aziz et al., 2013), increase plant growth and suppress the development of disease in plants (Gusnawaty HS et al., 2013). Apart from the disease problem of soybean cultivation in the field is also constrained by the problem of low soil fertility, so the need for the addition of nutrients by using organic materials such as soybean and rice straw around us that are often not used or wasted. Therefore, it is very possible for organic material to be processed into a bokashi with a decomposer. In general, farmers use EM4 as an organic decomposer and have been commercialized. This research focuses on the effectiveness of the bokashi decomposer and dosage of inorganic fertilizer in increasing the growth and resistance of soybean plants to disease, in addition to comparing the effectiveness of decomposer *Trichoderma* sp. and EM4.

II. MATERIALS AND METHODS

The research was carried out in the Experimental Garden of the Faculty of Agriculture and the Laboratory of Plant Protection, Halu Oleo University. The research design was used namely a Randomized Block Design (RBD) factorial pattern. The first factorial is a fertilizer with different of bokashi decomposers (B) consisting of 4 (four) levels, namely; B0: without decomposer, B1: effective Microorganism-4 (EM4), B2: *Trichoderma asperellum*, B3: effective Microorganism-4 (EM4) + *Trichoderma asperellum*.

The second factor is the dose of inorganic fertilizer (A) consisting of five levels, namely; A0: without inorganic fertilizer, A1: 25% of the recommended dose of inorganic fertilizer, A2: 50% of the recommended dose of inorganic fertilizer, A3: 75% of the recommended dose of inorganic fertilizer, A4: 100% of the recommended dose of inorganic fertilizer. So that there can be 20 treatments combination, each treatment is repeated three times so that there are 60 treatments in total.

All of the data were obtained analyzed by analysis of variance (ANOVA) and if the treatment had a significant effect based on the F test, then a further test was carried out by comparing the average value of treatment with the Duncan Multiple Range Test (DMRT) at α: 0.05.

III. RESULTS AND DISCUSSION

Results

Plant height

Table 1. The main effects of different bokashi decomposers (B) on soybean plant height (cm) two, four, six and eight weeks after planting (WAP)

Bokashi decomposers (B)	Average of Plant Height			
	2 WAP	4 WAP	6 WAP	8 WAP
(B0)	10.24 b	18.40 c	34.93 b	45.64 c
(B1)	11.24 a	20.11 b	44.13 a	55.15 b
(B2)	11.09 a	21.60 a	44.79 a	59.60 a
(B3)	10.83 ab	21.63 a	46.03 a	58.33a
DMRT 5%	2=0.6140 3=0.6455 4=0.6661	2=0.8445 3=0.8879 4=0.9163	2=2.124 3=2.233 4=2.304	2=3.112 3=3.271 4=3.376

The result of DMRT (Table 1) showed that the highest average of the main effect of organic decomposer was found at 8 WAP namely the B2 (*T. asperellum*) of 59.60 which was not significantly different from B3 (*T. asperellum* + EM4 decomposer) but significantly different with other treatments.

Table 2. The main effects of dose inorganic fertilizer (A) on soybean plant height (cm) six and eight weeks after planting (WAP).

Doses ofinorganic fertilizer (A)	Average of Plant Height	
	6 WAP	8 WAP
(A0)	38.83 c	51.03 c
(A1)	40.97 bc	52.80 bc
(A2)	42.78 ab	54.92 ab
(A3)	44.50 a	56.92 a
(A4)	45.27 a	57.73 a
DMRT 5%	2=2.374 3=2.496 4=2.576 5=2.633	2=3.479 3=3.658 4=3.774 5=3.858

The result of the DMRT (Table 2.) showed that the average of the main effect of inorganic fertilizer dose at 6 and 8 WAP was found in treatment A4 (100% of the recommended dose of inorganic fertilizer) which was not significantly different from A3 (75% of the recommended dose of inorganic fertilizer) and A2 (50% of the recommended dose of inorganic fertilizer), but significantly different from A1 (25% of the recommended dose of inorganic fertilizer) and A0 (without inorganic fertilizer). A2 is not significantly different from A1 but significantly different A0, while A1 is not significantly different from A0.

Number of Leaves

Table 3. The main effect of different types of bokashi decomposers on the number of leaves (cm) of soybean plants six and eight weeks after planting (WAP).

Bokashi decomposers (B)	Average of Number of Leaves	
	6 WAP	8 WAP
(B0)	8.87 b	12.79 b
(B1)	12.96 a	17.87 a
(B2)	13.13 a	17.99 a
(B3)	12.56 a	16.84 a
DMRT 5%	2=0.7452 3=0.7835 4=0.8085	2=1.311 3=1.378 4=1.422

DMRT follow-up test result (Table 3.) showed that the highest average of themain effect of agricultural waste decomposers (B) was found at 6 and 8 WAP inB2 (*T. asperellum*decomposer) which is not significantly different from B1 (EM4 decomposer) and B3 (*T. asperellum* + EM4 decomposer) but significantly different from B0 (without decomposer).

Table 4. Effect of the main dose of inorganic fertilizer on the number of leaves of soybean plants six and eight weeks after planting (WAP).

Doses of inorganic fertilizer (A)	Average of Number of Leaves	
	6 WAP	8 WAP
(A0)	9.58 e	13.00 d
(A1)	10.72 d	14.67 c
(A2)	11.67 c	15.93 c
(A3)	12.75 b	17.83 b
(A4)	14.68 a	20.42 a
DMRT 5%	2=0.1180 3=0.1241 4=0.1280 5=0.1309	2=1.466 3=1.541 4=1.590 5=1.626

DMRT test results (Table 4.) showed that the highest average of the main effect of the dose of inorganic fertilizer (A) was found at 6 WAP in A4 (100% of the recommended dose of inorganic fertilizer) of 14.68 which was significantly different from other treatments. The lowest was found in A0 (without inorganic fertilizer) of 9.58. The highest value at 8 WAP namely A4 (100% of the recommended dose of inorganic fertilizer) of 20.42 which is significantly different from other treatments. A3 (75% of the recommended dose of inorganic fertilizer) is significantly different from A2 (50% of the recommended dose of inorganic fertilizer) but A2 is not significantly different from A1 (25% of the recommended dose of inorganic fertilizer) and significantly different from A0.

Disease incidence

1. Soybean Mosaic Virus (SMV)

Based on field observations, the initial appearance of disease symptoms soybean mosaic virus (SMV) in soybean plants was found at 6 WAP and continued to develop up to 10 WAP. Symptoms of soybean mosaic virus (SMV) found in the field are as shown below:

Figure 1. Symptoms of soybean mosaic virus (CPMMV) disease

Table 5. The main effect of different types of bokashi decomposers on SMV disease incidence of soybean plants six, seven, eight, nine, and ten weeks after planting.

Bokashi decomposers (B)	Weeks After Planting (WAP)				
	6	7	8	9	10
B0	7.22 a	9.95 a	14.29 a	18.01 a	23.15 a
B1	5.80 b	8.80 a	12.57 a	15.99 a	19.63 b
B2	3.87 c	6.93 b	10.45 b	12.52 b	16.28 c
B3	5.97 ab	9.03 a	12.82 a	16.20 a	20.11 b
DMRT 5%	2=1.285	2=1.688	2=2.078	2=2.517	2=2.962
	3=1.352	3=1.774	3=2.184	3=2.647	3=3.114
	4=1.395	4=1.831	4=2.254	4=2.731	4=3.213

=The results of DMRT tests (Table 5.) showed that the lowest of the main effect of different types of bokashi decomposers (B) on the SMV disease incidence was found in B2 (*T. asperellum*) treatment at 6, 7, 8, 9 and 10 WAP, respectively 3.87, 6.93, 10.45, 12.52 and 16.28, which are significantly different from other treatments.

Table 6. The main effect dose of inorganic fertilizer on SMV disease incidence of soybean plants six, seven, eight, nine, and ten weeks after planting.

Doses of inorganic fertilizer (A)	Weeks After Planting (WAP)				
	6	7	8	9	10
A0	8.58 a	12.41 a	16.93 a	21.13 a	26.12 a
A1	6.36 b	9.82 b	14.24 b	17.64 b	21.88 b
A2	6.45 b	9.56 b	13.71 b	17.84 b	21.93 b
A3	4.06 c	6.63 c	10.06 c	12.77 c	17.11 c
A4	3.12 c	4.96 c	7.72 d	9.01 d	11.92d
DMRT 5%	2=1.437	2=1.887	2=2.323	2=2.815	2=3.311
	3=1.511	3=1.984	3=2.442	3=2.959	3=3.482
	4=1.559	4=2.047	4=2.520	4=3.054	4=3.593
	5=1.594	5=2.093	5=2.576	5=3.122	5=3.673

=DMRT test results (Table 6,) showed that the lowest of the main effect of doses inorganic fertilizer (A) was found in A4 (100% of the recommended dose of inorganic fertilizer) treatment at 6, 7, 8, 9 and 10 WAP respectively 3.12, 4.96, 7.72, 9.01 and 11.92 which were significantly different from other treatments.

2. Cowpea Mild Mottle Virus (CPMMV)

Cowpea mild mottle virus (CPMMV) disease appears on soybean plants at six weeks after planting with curved yellow symptoms on leaves. CPMMV symptoms found in the field are as shown below:

Figure 2. Symptoms of Cowpea mild mottle virus (CPMMV) disease

Table 7. The main effect of different types of bokashi decomposers on CPMMV disease incidence of soybean plants seven, eight, nine and ten weeks after planting.

Bokashi decomposers (B)	Weeks After Planting (WAP)			
	7	8	9	10
B0	5.14 a	7.37 a	10.03 a	15.44 a
B1	4.78 a	7.05 ab	9.97 a	11.80 a
B2	3.58 b	4.71 b	5.54 c	7.75 b
B3	4.35 ab	5.56 b	7.30 b	8.76 b
DMRT 5%	2=1.061 3=1.115 4=1.151	2=1.539 3=1.618 4=1.67	2=1.688 3=1.775 4=1.832	2=1.987 3=2.090 4=2.156

The result of DMRT test (Table 7.)showed that the lowest of the main effect of different types of bokashi decomposers (B) on CPMMV disease incidence of diseases was found in B2 (*T. asperellum*) treatment at 6, 7, 8, 9 and 10 MST respectively 3, 58, 4.71, 5.54 and 7.75 which were significantly different from other treatments.

Table 8. The main effects of inorganic fertilizer doses on CPMMV disease incidence of soybean plants six, seven, eight, nine, and ten weeks after planting.

Doses of inorganic fertilizer (A)	Weeks After Planting (WAP)				
	6	7	8	9	10
A0	3.93 a	5.52 a	7.60 a	9.38 a	13.02 a
A1	3.05 ab	4.59 a	5.95 a	7.97 ab	10.96 b
A2	2.81 b	4.81 a	6.90 a	9.11 a	12.13 ab
A3	2.33 b	4.34 a	6.19 a	8.15 ab	10.53 ab
A4	1.76 b	3.06 b	4.22 b	6.43 b	8.04 c
DMRT 5%	2=1.073 3=1.128 4=1.164 5=1.19	2=1.186 3= 1.247 4=1.287 5=1.315	2=1.721 3=1.809 4=1.867 5=1.908	2=1.888 3=1.985 4=2.048 5=2.094	2=2.222 3=2.336 4=2.411 5=2.464

The result of DMRT test (Table 8.) showed that the lowest of the main effect of inorganic fertilizer doses of CPMMV disease incidence was found in the A4 treatment at 6, 7, 8, 9 and 10 MST respectively 1.76, 3, 06, 4.22, 6.43, and 8.04 were significantly different from other treatments.

Area Under Disease Progress Curve (AUDPC) Disease Incidence and Suppression

1. Soybean Mosaic Virus (SMV)

Table 9. The main effect of different types of bokashi decomposers (B) on the AUDPC SMV disease incidence and AUDPC SMV disease suppression of soybean plants.

Bokashi decomposers (B)	AUDPC Disease Incidence	AUDPC Disease Suppression (%)
B0	779.66 a	0
B1	669.96 b	14.07
B2	539.98 c	30.74
B3	684.81 ab	12.17
DMRT5%	2=100.1 3=105.3 4=108.6	

The result of the DMRT test (Table 9.) showed that the lowest of AUDPC value of SMV disease incidence was found in B2 (*T. asperellum*) treatment of 539.98 and the highest of disease suppression also in B2 of 30.74%.

Table 10. The main Effect of inorganic fertilizer doses on AUDPC SMV disease incidence and disease suppression of soybean plants.

Doses of inorganic fertilizer (A)	AUDPC Disease Incidence	AUDPC Disease Suppression (%)
A0	898.97 a	0
A1	747.38 b	16.86
A2	741.83 b	17.48
A3	558.19 c	37.91
A4	396.65 d	55.88
DMRT 5%	2=111.9 3= 117.7 4=121.4 5=124.1	

The result of the DMRT test (Table 10.) showed that the lowest of AUDPC value of SMV disease incidence was found in A4 (100% of the recommended dose of inorganic fertilizer) treatment of 396.65 and the highest of disease suppression also in A4 of 55, 88%.

2. Cowpea Mild Mottle Virus (CPMMV)

Table 11. The main effects of different types of bokashi decomposers on AUDPC CPMMV disease incidence and disease suppression of soybean plants.

Bokashi decomposers (B)	AUDPC Disease Incidence	AUDPC Disease Suppression (%)
B0	456.72 a	0
B1	419.24 a	8.21
B2	270.17 b	40.85

B3		323.24 b	29.23
DMRT 5%	2=70.30 3=73.91 4=76.27		

The result of the DMRT test (Table 11.) showed that the lowest of AUDPC value of CPMMV disease incidence was found in B2 (*T. asperellum*) treatment of 270.17 and the highest of disease suppression in B2 of 40.85%.

Table 12. The main effect of doses of inorganic fertilizer on AUDPC CPMMV disease incidence and disease of soybean plants.

Doses of inorganic fertilizer (A)		AUDPC Disease Incidence	AUDPC Disease Suppression (%)
A0		429.63 a	0
A1		371.20 a	13.60
A2		410.38 a	4.48
A3		360.46 a	16.10
A4		265.04 b	38.31
DMRT 5%	2=78.59 3= 82.63 4= 85.27 5=87.17		

The result of the DMRT test (Table 12.) showed that the lowest of AUDPC value of CPMMV disease incidence was found in A4 (100% of the recommended dose of inorganic fertilizer) treatment of 258.88 and the highest of disease suppression also in A4 of 38.31%.

Discussion

Biofertilizer made from active living organisms in liquid or solid form which has the ability to mobilize, facilitate and increase the availability of nutrients that are not available such as nitrogen (N_2) nutrient bound in minerals or bound in the form of organic compounds into a form available through biological processes. Decomposers are categorized as bio-fertilizers because they play an active role in converting non-available nutrients into available nutrients through the decomposition process (Board, 2012; Singh and Purohit, 2011).

Trichoderma sp. is a saprophytic soil microorganism that naturally attacks pathogenic fungi and beneficial for plants. *Trichoderma* sp. is one type of fungus that is found in almost all types of soil and in various habitats which is one type of fungus that can be used as biological agents controlling of soilborne pathogens and be able to use as a decomposer. Novizan (2005) states that small amounts of nutrients derived from biofertilizers can be used directly by plants, but some are broken down over a long period of time. With the help of microorganisms in the soil, organic matter will be converted into a simple form and absorbed by plants (Musnawar, 2005). Therefore, biofertilizer must undergo a perfect decomposition before it is available to plants in the soil. This state was proven by Ismail and Andi (2011) who stated that the use of *Trichoderma* sp. can break down nutrients that are in the soil, produce glycotoxic and viridian antibiotics that can be used to protect plants from disease, in addition, be able to release enzymes β-1,3-glucanase and chitinase which can dissolve pathogen cell walls.

Based on observations on the field in this research was found aphids as a virus vector under the surface of leaves, flowers, ovaries, and in folds of curly leaves. Those aphids have a characteristic green body or pale green, sometimes orange or yellow. This vector becomes a reference that the symptoms that appear in the soybean plant caused by a virus. Viruses can be transmitted non-persistently through seeds and can be transmitted mechanically by several types of aphids such as *Aphis glycines*, *A. craccivora* and *Myzus persicae* (Nazir, 2005). Viral infection in soybean plants occurs if the virus through various ways such as smooth injury and insect then enters the cell and multiplication. Multiplication of RNA and virus coat protein occurs separately which eventually unite to form new virus particles. Viral systemic infections allow the entry of viruses into the seeds that occur through infection of the ovum and pollen (Rachmadi et al. 1987; Saleh et al. 1987).

Figure 1. shows symptoms of the bone of leaves in young leaves become clear yellow. After that the leaves become shrink, the edge of leaves becomes curved, the leaves bone thickens, curved inward and outward. Soybean mosaic virus (SMV) infection causes symptoms in plants, such as leaf surface is uneven, wrinkled, leaves shrink with mosaic, rolled in, and chlorosis, sometimes plants becoming stunted and dotted symptoms (Andayanie et al., 2012). ELISA test results that have been done by previous researchers Anwar (2015) found symptoms of disease caused by SMV in soybean plants in Southeast Sulawesi and have similar symptoms of disease with observations that have been made in this research (Figure 1).

Figure 2. shows symptoms of dwarf plants, yellowing of leaves, blanching of leaves, and distortion of leaves or curved leaves. Symptoms of CPMMV infection in soybean plants are systemic stripes, leaf bone bleaching, and leaf distortion in soybean plants (Mimi et al., 2017). ELISA test results conducted by Taufik et al., (2015). prove that there are three types of viruses that cause multi-infection in soybean plants in Southeast Sulawesi, namely Cowpea mild mottle virus (CPMMV), Cucumber mosaic virus (CMV) and Soybean mosaic virus (SMV).

Based on the results of the independent treatment of bokashi decomposers (B) and the dose of inorganic fertilizer (A) on the incidence of the diseases caused by SMV and CPMMV there was no interaction. This shows that *Trichoderma asperellum* treatment can reduce the incidence of diseases by inducing plant resistance. *Trichoderma* sp. is a saprophyte soil microorganism that is naturally beneficial for plants (Gusnawaty HS et al., 2014). Furthermore, based on observations showing that the higher disease incidence happened in the treatment without inorganic fertilizer (A0). Inorganic fertilizers such as NPK fertilizers are important and be able to influences the growth and suppress the development of plant diseases. NPK fertilizers are involved in the process of plant defense against pathogens and helping activity of biocontrol agents, especially in interactions between nutrients, pathogens, and biocontrol organisms (Wasis and Praptana, 2013), but excess or deficiency of NPK can cause the accumulation of free amino acids in plant tissue and be able to stimulate insect populations and pathogens that cause damage to plants (Leite et al, 2012).

The results of the AUDPC calculation of disease incidence caused by SMV and CPMMV viruses with bokashi decomposers (B) and inorganic fertilizer doses (A) have not interacted. The lowest average AUDPC value in diseases incidence caused by CMV and CPMV of soybean plants is in the treatment with *Trichoderma asperellum* (B2) respectively 539.98 and 270.17, while for inorganic fertilizer doses (A), the lowest average AUDPC value in diseases incidence caused by CMV and CPMV of soybean plants is in the treatment with 100% of the recommended dose of inorganic fertilizer (A4) respectively 396.65 and 265.04. These results were significantly different from no bokashi decomposers (B0) and without inorganic fertilizer (A0). This indicates that the use of *Trichoderma asperellum* (B2) as a decomposer for agricultural waste bokashi and 100% of the recommended dose of inorganic fertilizer (A4) can reduce the incidence of the diseases caused by the SMV and CPMMV in soybean plants. Applying biofertilizers on ultisol marginal land can increase crop resilience by inducing soybean crop resistance to diseases caused by viruses (Iwaki, 1980). Increased plant growth by adding of biological agents such as *Trichoderma* sp., *T. harzianum, T. koningii, T. viridae* are able to stimulate plants to produce gibberellic acid (GA3), indolasetat acid (IAA), and benzylaminopurin (BAP) in a larger amount, so that the plant growth is more optimum, fertile, healthy, sturdy, and ultimately affects the plant resistance (Eli et al., 2015). Biofertilizer and inorganic fertilizer have important roles in soybean plant, but biofertilizer is more recommend to used because biofertilizer does not negatively impact in the soil and plants if applied continuously, different with inorganic fertilizer, therefore to get optimal results, inorganic fertilizer must be given in sufficient quantities to the needs of plants, not excessive and not less (Eli et al., 2015).

IV. CONCLUSION

Based on the results of research, it can be concluded that there is no interaction effect of the type of decomposer of agricultural waste with inorganic fertilizer fertilizers on the growth and disease incidence caused by viruses in soybean plants. B2 (*Trichoderma asperellum*) is the best main treatment of bokashi decomposers that increase plant growth and has the effectiveness to suppress CMV and CPMMV diseases respectively 30.74% and 40.85%, while A4 treatment is the best dose of inorganic fertilizer with 100% of the recommended dose of inorganic fertilizer that effective in increasing plant growth and suppressing CMV and CPMMV diseases which caused by viruses respectively 55.88% and 38.31%.

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