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Effect of biological agents (biofresh) and organic matter to progress of soybean mosaic virus (smv) and yield of soybean in Sub-Optimal Ultisol land

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Abstract

Soybean mosaic virus (SMV) is most recognized as the source of problems for the soybean crop. This study aimed to investigate the effect of biological agents (Biofresh) and organic matter on the progress of Soybean Mosaic Virus (SMV). This study uses factorial randomized complete block design consisting of two factors i.e. biological agent (Biofresh) formulations and organic matter compost treatments. Data were analyzed using the SAS 9.1.3 version statistical softwere. The treatments were significantly different tested further by Duncan multiple range test at 5% level. Single treatment of Biofresh formulations and the type of organic matter influenced the latent period, the disease severity, AUDPC value of SMV and the soybean plants grown in sub-optimal ultisol land. The latent period symptoms of SMV applied with Biofresh formulation and organic matter were found on average of 9-11 days after inoculation. Plant height and number of leaves of soybean plants cultivated with the combination treatment of solid formulations and compost of soybean litter waste (A1C1) showed the best results, with plant height and number of leaves at 10 WAP are 49.37 cm and 26.53 sheets. Plants treated with Biofresh of solid formulation and organic matter soybean (A1C1) showed the best production with crop yields 261.00 pods, 644.33 seeds and 92.17 g seed weight. Organic matter in a solid formulations that is applied with organic matter of soybean litter waste can increase the growth and sustainability of soybean and soybean crop yields.

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Introduction

Soybean mosaic virus (SMV) is the most famous virus that cause problems in soybean crops in all regions.SMV infection causes the loss of 50-90% yield and reduce nitrogen fixation, seed size and oil content (Ross 1969; Demski and Jellum 1975; Cho *et al.,* 1977; Dhingra and Chenulu 1980; El-Amrety *et al.,* 1985; Giesler *et al.,* 2010). Higher loss of yield could be occurred if the infection occurs onthe soybean crop that cultivated in sub-optimal ultisol land. Development of SMV is strongly influenced by the fertility of the land, therefore the improvement of fertility and nutrient content are very important in increasing soybean production and plant resistance to disease in sub-optimal ultisol land.

Some efforts should be done to increase production of soybean as well as to increase the resistance of soybean plants to SMV. Two of these efforts are using Plant Growth Promoting Rhizobacteria (PGPR) and organic materials. PGPR can act as bioprotectant and biostimulant that can serve to depress and inhibit the growth of pathogens, and improve plant growth and crop production. PGPR is a group of bacteria that comes from different genera such as *Bacillus*, *Azotobacter*, *Azospirillum* and *Serratia* (Tailor and Joshi, 2014). *Bacillus* sp. can act as strong of biocontrol agent because it can suppress phytopathogenic fungi by producing the lipopeptide antibiotics, extracellular enzymes and siderophores (Hassan *et al.,* 2010; Ait-Kaki *et al.,* 2014). Based in this information, the development of PGPR to improve soybean resistance of SMV and soybean production in sub-optimal ultisol land is very required.

Biological agents (Biofresh) is a biological fertilizer formulation containing a mixture of three strains PGPR, namely: *Bacillus cereus* ST21b, *B. subtilis* ST21e and *Serratia sp* SS29a, Biofresh is mixture of biological agent that has the ability to stimulate the growth of food crops and horticulture. The use of (Biofresh) biological agents with inorganic fertilizers up to 50% of the recommended dose can improve soybean plant resistance to SMV and bacterial leaf blight (Khaeruni *et al.*, 2010; Khaeruni *et al.*, 2015). Metabolite products produced by PGPR as contained in Biofresh can directly suppress the pathogens or by induced systemic resistance (ISR) mechanism in plants (Hassan *et al.,* 2015). Induced systemic resistance is the state of the wide spectrum of defense capability of plants associated with jasmonic acid and ethylene signaling, elicited by various biotic and abiotic factors (Shoresh *et al.,* 2005; Kwon *et al.,* 2009). This enhances the response of plant to innate immunity and to accelerate expression of defense to related enzymes (Van der Ent *et al.,* 2009).

The use of biological control agents to control the pest is more developed because this method is considered more superior than pesticide-based control. Biological agents have the advantages that are safe for humans, prevent of pest and plant products are free of pesticide residues. Organic matters have direct role to the nutrient needs of plants that support various of metabolic processes. Therefore, this research will report the impact of biological agents (Biofresh) formulation and organic matter on progress of SMV and soybean crops in sub-optimal ultisol land.

Materials and methods

Soybean seeds were obtained from Indonesian Legumes and Tuber Crops Research Institute, Indonesian Agency for Agricultural Research and Development, Malang, Indonesia.*Bacillus cereus* ST21b, *B. subtilis* ST21e and *Serratia sp* SS29awere obtained from Biomolecular Laboratory, Halu Oleo University. Silicon carbide was purchased from Aldrich, Singapore.

Experimental design

This study uses factorial randomized complete block design consisting of two factors. The first factor is a biological agent formulations (Biofresh) which consists of three levels i.e.: A0 = without Biofresh (control), A1 = solid formulations, A2 = liquid formulations.The second factor is the treatment by using organic matter compost consisting of four levels i.e.: C0 = Without organic matter, C1 = compost of soybean litter waste, C2= compost of rice straw, C3 =

compost of soybean litter waste + rice straw. Overall, there are 12 combinations of treatments, each treatment was repeated 3 times so that there are 36 units of treatment and each treatment consist of 5 units of the sample, so that the whole sample contained 180 units of the plant. Data were analyzed using the SAS 9.1.3version statistical softwere. The treatments were significantly different tested further by Duncan multiple range test at 5% level.

Preparation growing media and cultivation of soybean seeds

The growing media used in this research is ultisol soil that has been sterilized with vapor sterilization. Soil thoroughly mixed with straw compost, soybeans compost and manure according to treatment with a ratio of 1:7. The mixture were then placed in a polybag with size of 30 × 40 cm and placed on open land at Experimental Farm, Faculty of Agriculture, Halu Oleo University, Kendari according to design of experiments.

Three of soybean seeds were cultivated at depth of 5 cm from the surface of growing media. Two weeks after cultivating, two plants were selected and were retained as a plant sample, while the rest is removed and discarded.

Formulation and application of Biofresh

Before mixing with material formulation, *Bacillus cereus* ST21b, *B. subtilis* ST21e and *Serratia* sp SS29a rhizobacteria were propagated on solid TSA medium separately and were incubated for 48 h. Bacteria colonies growth was suspended in sterile distilled water until it reaches population density of 1010 cfu/mL and was used as raw material in the making of two formulations of Biofresh.

Solid formulation is prepared by mixing the third rhizobacteria suspension with carrier materials such as peat organic matter and animal manure with certain ratio. Furthermore, the mixture of biological agent and material formulation was dried for 48 h, was packed in plastic bags to 5 Kg and is ready for application. For the liquid formulation, it is prepared by mixing the third rhizobacteria suspension with a carrier material in the form of a mixture of water, coconut water and Tryptic Soy Broth with certain ratio in a plastic container 5 L, the mixture was then incubated for 48 h and was ready for application.

Applications of Biofresh was conducted during planting process. 4 WAP (Week After Plantation) was sprinkled withliquid formulation around the planting hole as much as 10 mL and solid formulations for covering planting hole during planting and was spreaded around 10 grams per planting hole at the age of 4 WAP.

Preparation and inoculation of sap inoculum SMV

The sap used the plants that positively infected by SMV that were kept in the screen house, Faculty of Agriculture, Halu Oleo University. Sap inoculum SMV was prepared by taking leaves of soybean plant that showed symptoms of SMV, then were washed and were cut into pieces. 5 g of leaves were crushed with mortar and were added with 10 mL of phosphate buffer (10 mM). Sap was obtained by filtering using gauze.

Contagion of sap SMV was done mechanically on the young leaves of soybean aged 21 days after planting. The top surface of soybean leaves was sprinkled with 600 mesh silicon carbide then was applied uniformly by sap SMV. Leaf surface was uniformly sprayed with distilled water using hand sprayer.

Observation variabels

Observations were carried out on five samples of plants from each treatment unit, the variables observed are:

(1) The latent period and disease symptoms: Observations carried out from the starting day (the day one) after inoculation until the appearance of the first symptom in all treatments.

(2) Disease severity: SMV disease severity was calculated using the scoring method through assessment score of the plant leaves ill based on symptoms mosaic formed in plant samples (Hassan *et al.,* 2015). Score category of SMV attack on the leaves were: (0) healthy leaves (do not show symptoms); (1)

mosaic symptoms <50% of the leaves area; (2) mosaic symptoms> 50% of the leaves area; (3) mosaic symptoms and leaf size was reduced; (4) mosaic symptoms with smaller leaves and wrinkled; (5) mosaic symptoms with smaller leaves size and wrinkled and roll leaves.

Scoring result was then used to calculate the severity of the disease by using the formula (equation 1):

$$DS = \sum_{i=0}^{n} \left(\frac{n.V}{Z.N}\right) x\ 100\ \% \text{ ....(1)}$$

DS: disease severity (%); n: the number of leaves attacked in each category; N: the number of leaves that were observed; V: value scale of each category of that were attacked and Z: the highest scale value of attacked category.

Besides disease severity values, the value of area under disease progress curve (AUDPC) was also calculated to observe the disease progress. Values was calculated based on the formula AUDPC Van der Plank (Cooke, 1998), equation 2:

$$\text{AUDPC} = \sum_{i=1}^{n=1} \left(\frac{y_i + y_{i+1}}{2}\right) x\ (t_{i+1} - t_i) \text{ ....(2)}$$

In this method, we stat with disease severity (or incidence) data (yi) collected at various times (t_i).

(3) Plant growth: Observations of plant growth was conducted on plant samples at the age of 10 WAP, in terms of plant height (cm) measured from the ground in polythene bags until the tip of leave, and the number of unfoldedplant leaves (strands) and productive branches.

(4) Crop yields: Crop yields was observed when the plants has been in mature phase of physiology which is characterized by yellowing of the leaves as much as 80%, variables of crop yields observed include: number of crop pods, the number of crop seeds, crop seed weight.

Results and discussion

The latent period and Infection symptoms of Soybean Mosaic Virus

The emergence of the first symptoms or infection in the latent period of Soybean mosaic virus (SMV) varies between 3-11 days after inoculation. Firstly, the symptomatic leaves possesses chlorosis, then the color changes from green to yellow with wrinkled leaves and roll up.Plants without treatment of biological agents (Biofresh) and organic matter (A0C0) and the treatment of biological agents liquid formulations without organic matter (A2C0) has the fastest of latent period i.e. 3 days after inoculation, while the longest of latent period found in treatment solid formulations and compost of soybean litter waste (A1C1) i.e. 11 days after inoculation (Table 1).

Table 1. Average latent period of SMV soybean crop.

Treatments	Incubation period (Day after inoculation)
A0C0	3
A0C1	6
A0C2	6
A0C3	8
A1C0	3
A1C1	11
A1C2	9
A1C3	10
A2C0	3
A2C1	9
A2C2	9
A2C3	9

Single treatment of biological agents (Biofresh), Biofresh and type of organic matter influence the latent period and disease severity of Soybean mosaic virus (SMV).The latent period is characterized by the appearance of disease symptoms in plants inoculated inoculum SMV. The symptoms are symptomatic leaves mosaic, malformation, cupping, wrinkled and yellowing of the leaves. These symptoms shows similar result with report by Han *et al.* (1970) and Kuroda *et al.* (2010) that report the virus in soybean were found typically mosaic, leaves surface uneven, thickening of bone leaves, malformation leaves, cupping and yellowing.

Table 2. Analysis result of biological agent and the type of compost organic matter formulations of disease severity SMV and AUDPC value.

Treatments	Disease Severity (%) on n WAI				AUDPC (unit)
	2	3	4	5	
Formulation of Biofresh (A)	**	**	**	*	**
Compost of Organic Matter (C)	*	**	**	**	**
Combination of AC	Ns	ns	Ns	ns	ns

The latent period symptoms of SMV was appeared in soybean plants that was applied with Biofresh formulations and organic matter in average of 9-11 days after inoculation, while the control plants symptoms of latent period at all test plants was appeared in average of 3 days after the inoculation. These results indicate the addition of biological agentsand organic matter are able to slow down the latent period of SMV in soybean. The same results have been reported by Hassan *et al.* (2015) that the latent period of the plants treated with PGPR more slowly than in plants without PGPR treatment.

Disease severity of Soybean Mosaic Virus (SMV)

Combination treatment of Biofresh and compost of organic matter formulations has no effect on disease severity of SMV, but Biofresh single treatment formulations was highly significant ($p \leq 0.01$) at 2, 3, 4, and significant ($p \leq 0.05$) at the age of 5 weeks after inoculation.

Table 3. Effects of Biofresh formulation of disease severity SMV on soybean.

Treatments (Formulation of Biofresh)	Disease severity on n WAI				AUDPC
	2WAI	3WAI	4 WAI	5 WAI	(% unit)
Control, Without Biofresh (A0)	27.33^{a}	34.33^{a}	38.72^{a}	41.66^{a}	752.85^{a}
Solid Formulation of Biofresh (A1)	23.00^{b}	28.66^{b}	33.33^{b}	36.33^{b}	641.66^{c}
Liquid Formulation of Biofresh (A2)	24.33^{b}	33.33^{a}	36.33^{ab}	37.33^{b}	703.49^{b}

Single treatment of compost oforganic mattershowedsignificant effect ($p \leq 0.05$) at 2 weeks after inoculation and highly significant ($p \leq 0.01$) at 3, 4, and 5 after inoculation. Highly significant effect ($p \leq 0.01$) is also indicated by AUDPC value on a single treatment ofbiological agents (Biofresh) and the type of compost of organic matterformulations (Table 2). Further test results showed that the average disease severity of SMV on a single treatment biological agents (Biofresh) formulations was not significantly different between the solid and liquid formulations, but both were significantly different from controls. Value severity of the disease in each treatment continue to grow along with the aging of plants with different levels of severity disease.

At the age of 5 weeks after inoculation, disease severity and the highest of AUDPC value were found in the control plant i.e. 41.66% and 752.85% units, at the same time, disease severity of SMV and AUDPC in the treatment of solid formulation were36.33% and 641.66 % unit which did not differ significantly with disease severity and AUDPC value in the treatment of liquid formulation i.e. 37.33% and 703.49% units (Table 3).

The results showed that the application of biological agents (Biofresh) in solid form by compost of soybean litter waste has higher ability to improve plant resistance to SMV in ultisol land, so the progress of the disease is slower than other treatments.In the treatment of a single type of organic matter, in the early observations, the disease severity of SMV in each treatment was varied between 23.11%-25.77% and continued to increase until the end of the observation (Table 4).

Table 4. Effects of organic matter of severity disease SMV on soybean.

Treatments (Organic Matter Compost)	Disease severity on n WAI				AUDPC (% unit)
	2 WAI	3 WAI	4 WAI	5 WAI	
Control, Without compost (B0)	26.66^{a}	36.00^{a}	41.33^{a}	42.66^{a}	784.00^{a}
Soybean litter waste compost (B1)	23.11^{c}	27.11^{c}	31.18^{c}	32.00^{b}	600.95^{c}
Rice straw compost (B2)	25.77^{ab}	33.77^{ab}	37.33^{b}	40.44^{a}	729.53^{b}
Mix soybean litter and Rice straw compost (B3)	24.00^{bc}	31.55^{b}	34.66^{bc}	38.66^{a}	682.88^{b}

At the age of 5 weeks after inoculation, the disease severity of SMV and the highest of AUDPC value in control plants were 42.67% and 784% units respectively and the two values were significantly different from the treatment of compost of soybean litter waste. The treatment of compost of soybean litter waste has disease severity of SMV and has the lowest of AUDPC value i.e.32.00% and 600.96% units, respectively. Both values are significantly different from the disease severity and AUDPC treated with other treatments.

In the treatment of organic matter independently, treatment without organic matter (C0) continues to increase with the highest of disease severity at age 9 WAP of 52.89%, while the treatment of compost of soybeans litter waste (C1) at the same time was only by 35.11%. Organic matter can increase the activity of microorganisms colonize area of soybean crop rhizosphere so that the system can induce plant resistance to pathogen infection, thus the disease progression SMV is inhibited.

Table 5. Combination effects of Biofresh and organic matter formulations.

Treatments	Plant height (cm)	Number of leaves (sheet)
0	18.55^{e}	4.13^{e}
A0C1	46.13^{abc}	21.00^{bc}
A0C2	43.47^{bc}	20.60^{bc}
A0C3	44.00^{bc}	22.13^{bc}
A1C0	31.90^{d}	9.68^{d}
A1C1	49.37^{a}	26.53^{a}
A1C2	42.07^{c}	24.60^{ab}
A1C3	45.61^{abc}	18.40^{c}
A2C0	19.71^{e}	4.93^{e}
A2C1	42.90^{bc}	21.77^{bc}
A2C2	42.33^{d}	18.27^{c}
A2C3	46.96^{ab}	23.47^{ab}

Meyer *et al.* (2005) reported that the inhibition of Cucumber Mosaic Virus infection in plants *Arabidopsis thaliana* and tobacco could be induced by salicylic acid compounds and antimycin A. Several other studies indicate that increasing of plant resistance to pathogens was induced by rhizobacteria associated with increasingaccumulation of salicylic acid compounds and peroxidase in plant tissues (El-Borollosy and Oraby, 2012; Hassan *et al.,* 2015).

Plant growth (plant height and total leaves)

Combination treatment of various formulations Biofresh with various types of organic matter significantly affected to plant height and number of leaves. Height and number leaves of soybean plants at the age of 10 WAP varied between treatments (Table 5). Treatment variations of formulations and types of organic matter at 10 weeks after planting are presented in Table 5.

Table 5 shows the highest plant at age 10 WAP obtained in combination treatment of biological agents in solid formulations with compost of soybean litter waster (A1C1) of 49.37 cm and is significantly different with all the treatment given with biological agent of Biofresh (A0) and formulations in liquid form (A2) at treatment without organic matter (C0). The lowest plant height was obtained on combination treatment without biological agents and organic matter (A0C0) i.e. 18.55 cm and were not significantly different with combination treatment of the liquid formulation and without organic matter (A2C0) of 19.71 cm.

Table 6. Interaction effect of Biofresh formulation and organic matter against number of pods, number of seeds and seed weight per plant.

Treatments	Pod Number	Seed Number	Seed Weight (g)
A0C0	13.67^{e}	24.67^{f}	3.80^{f}
A0C1	248.00^{ab}	598.00^{ab}	88.73^{a}
A0C2	203.00^{bc}	505.33^{bcd}	7393^{bcd}
A0C3	231.33^{abc}	569.67^{abc}	81.57^{abcd}
A1C0	64.67^{d}	143.67^{e}	19.23^{e}
A1C1	261.00^{a}	644.33^{a}	92.17^{a}
A1C2	220.67^{abc}	550.33^{abcd}	83.63^{abc}
A1C3	200.00^{bc}	497.33^{cd}	72.77^{bcd}
A2C0	22.67^{de}	43.67^{f}	5.97^{f}
A2C1	209.00^{bc}	519.00^{bcd}	71.90^{cd}
A2C2	187.33^{c}	466.33^{d}	69.30^{d}
A2C3	237.00^{abc}	575.67^{abc}	84.97^{ab}

Observations on the number of leaves indicates the plant having largest number of leaves at age 10 WAP was obtained in combination treatment of biological agents in solid formulations with compost of soybeans litter waste (A1C1) as much as 26.53 sheets. This value is significantly different from all treatments without formulations and liquid formulations without organic matter (A0C0 and A2C0) i.e. 4.13 and sheets 4.93, respectively. Plants in the treatment of A0C0 and A2C0 have the lowest number of leaves and significantly different from other treatments.

The observation of plant height and number of leaves of soybean plants in the combination treatment of solid formulations with organic materials of compost of soybean litter waste (A1C1) showed the best results with plant height and number of leaves on the observation of 10 WAP is 49.37 cm and 26.53 sheet, the treatment was significantly different from other treatments. The results of this study indicate that the application of biological agents (Biofresh) formulations in solid form and compost of soybean litter waste are able to increase the growth quality of the plants growth compared to the other treatments. Thakuria *et al.* (2004) reported that the ability of rhizobacteria as the plant growth promoters is indicated by the ability to provide and to mobilize the absorption of various nutrients in the soil as well as synthesizing and changing the concentration of various phytohormones. Rhizobacteria isolates in Biofresh formulations is capable of producing indole acetic acid, dissolving phosphate and nitrogen fixation freely (Khaeruni *et al.,* 2010).

Besides the addition of Biofresh formulation, the growth of soybean plant is also affected by the application organic matter as source of nutrients, especially N, P and K that balanced for plant growth and does not decrease rizobacteria populationin the soil. Ademir *et al.* (2009) reported that the land treated with organic biomass additions have a microbial and organic carbon higher than without any organic matter. Soybean plants treated with Biofresh solid formulations and compost of soybean litter waste showed the best height and number of leaves compared to other treatments. Soybean litter waste has C/N ratio that lower than non-legume plants, so microbes canmore quickly decompose into simpler compounds that can be used directly by plants. Therefore, plant growth is faster with compost of soybean litter waste than compost of rice straw treatments (Jusoh *et al.,* 2013).

Crop yields

The results of variance showed that the combination of treatment formulations and different types of organic matter effect on crop yields such as: number of pods, number of seeds and seed weight per soybeans plant grown in sub-optimal ultisol land (data is not shown). The observation of the number of pods plant, number of seeds, and the weight of seeds produced crops and Duncan test are presented in Table 6.

Table 6 showed an average the highest of number of pods, number of seeds and seed weight always obtained in combination treatment of biological agents solid formulations with compost of soybeans litter waste (A1C1) are 261.00 pods, 644.33 grains and 92.17 g per plant, crop yields on the A1C1 treatment was not significantly different among several other treatments with the treatment A1C2 and A0C3. Instead, the lowest yields are always presented in A0C0 treatment namely 13.67 pods, 24.67 seed number and 3.80 g seed of weight. Crop yields are not significantly different from crop yields treated with treatment of Biofresh liquid formulations without organic matter (A2C0). The obtained crops showed that the application organic matter plays important role in improving the productivity of soybean plants cultivated on sub-optimal ultisol land. Growing a healthy plant has implications for the crop yields, therefore the plants treated with Biofresh formulation of solid and organic matter soybean (treatment A1C1), also showed the best production, i.e.261.00 pods, 644.33 seeds and 92.17 g seed of weight. The results are consistent with results of previous studies that the addition of organic matter without composting of soybean litter waste can improve soybean plant resistance to disease pustules bacteria and promote the growth and production of soybean crops in the field (Khaeruni *et al.,* 2015). The use of biological agents as a biopesticide reported by Hassan *et al.* (2015) stated that the control of red rot disease of sugarcane proved to be very effective in the field.

Conclusion

From the results of this study, it can be concluded that organic matter in a solid formulations that is applied with organic matter of soybean litter waste can increase the growth and sustainability of soybean and soybean crop yields so that it can be recommended as a package cultivation in sub-optimal ultisol land.

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