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The effectiveness of a biotechnological-based fertilizer "Biofresh" in combination with organic matters on soybean health and production.

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ABSTRACT

Bacterial pustule disease is one of the most important disease problems of soybean. This study aimed to evaluate the role of various organic matters on the effectiveness of biotechnological-based fertilizer "Biofresh" as disease resistance inducer of soybean. "Biofresh" fertilizer in combination with different organic materials was applied on 2 soybean varieties. Research results showed that organic matters improved the biofresh effectiveness in increasing soybean yield and disease resistance. Soybean variety influenced the pustule disease development. Disease severity on Gema variety reached 44.23%, while on Argo Mulyo variety was only 11.09%. Application of soybean litter on Argo Mulyo variety reduced disease severity to 22.33%, significantly different from those of other treatments. Disease severity on treatment without organic material was 31.20%. Soybean litter in combination with biofresh allegedly gave better effect to Argo Mulyo variety in increasing plant production, which reached 1.54 tons/ha, a 77.1% increase compared to treatments without organic material. The biofertilizer "Biofresh" in combination with organic matter, especially soybean litter, can be applied to reduce the severity of soybean pustule disease and increase soybean production.

Keywords: bacterial pustule disease, biofertilizer, organic matter, soybean

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INTRODUCTION

Bacterial pustule disease caused by *Xanthomonas axonopodis* pv. *glycines* is one of the most important diseases of soybean in the tropics and subtropics [1, 2]. Yield loss caused by the disease in central areas of soybean planting in Indonesia reached 15.9% to 50% [3].

Despite numerous attempts to control pustule disease, e.g. by planting resistant varieties, mulching, and application of pesticides, no satisfactory results have been accomplished, because they were done partially [4]. Therefore, the control should be carried out in an integrated manner by combining several control techniques that are more effective, efficient, and environmentally friendly. Integrated control aims to optimize biological resources and reorganizing agriculture agro-ecosystem that is beneficial for biological agents and harm to pathogen, which is known as IPM - Biointensive.

Resistant varieties, biological agents, and agricultural waste organic materials can be integrated in an IPM - Biointensive of soybean in sub-optimal ultisol land. The use of rhizobacteria as biological agents have been reported to induce plant resistance to bacterial diseases in plant filosfer, including application of Plant Growth Promoting Rhizobacteria (PGPR) that was able to induce resistance of cucumber against bacterial wilt disease caused by *Erwinia tracheiphila* (Smith) [5]; mixture of 2 isolates of *Pseudomonas fluorecens* (Pf32 & Pf93) and *Bacillus subtilis* B49 effectively controlled bacterial blight of cotton both under greenhouse and field conditions [6], and the combined use of PGPR with straw mulch on “Gepak Kuning” variety effectively suppressed the development of soybean bacterial pustule disease caused by *Xanthomonas axonopodis* pv. *glycines* [4]. Application of *Bacillus* sp. and *Pseudomonas* sp. can suppress leaf blight disease caused by *Pantoea* sp on corn crops by 18% to 24% [7], and the application of indigenous rhizobacteria on seeds was capable of inducing resistance of rice plants to bacterial leaf blight caused by *X. oryzae* pv. *oryzae* [8, 9, 10].

Application of biological fertilizer Biofresh combined with 50% recommended doses of inorganic fertilizers N, P, and K also effectively increased the growth and yield, as well as induced soybean resistance against bacterial leaf blight caused by *Pseudomonas syringae* pv. *glycines* [11]. Biofresh is a biofertilizer which contains rizobacterias *Bacillus subtilis* ST21e, *B. cereus* ST21b and *Serratia* sp. SS29a which have the ability to produce phytohormones IAA, dissolve phosphate, and fix N, as well as capable of producing degrading enzymes such as chitinase, proteinase, and sellulase [12]. Application of biofresh with organic cow manure significantly increased the growth, yield, and soybean plant resistance against Rhizoctonia stem rot disease [13]. Application of organic materials is a promising soil management practice which can improve soil structure and increase soil microbial activity [14]. Organic materials such as rice straw, soybean litter, chicken manure and cow manure are potential to be used to improve the physical, chemical and biological soil properties to improve soil fertility [15, 16].

This study aimed to determine the effect of the application of biofertilizer Biofresh with different organic materials against bacterial pustule disease, growth and yield of two soybean varieties in sub-optimal ultisol land.

MATERIALS AND METHODS

The research was conducted on sub-optimal ultisol land (pH 4.9) at the Lamomea village, Konda District, Southeast Sulawesi, which was located at 4°11'51.29" South Latitude and 122°41'79" East Longitude. Soybean planting was done during a dry season, from August to November 2014, with average daily rainfall of 0 to 0.3 mm and an average daily temperature of 27°C to 28°C. The research location was an endemic area for soybean bacterial pustule disease caused by *Xanthomonas axonopodis* pv. *glycines*.

The research used Argo Mulyo and Gema soybean varieties, obtained from seed growers in Southeast Sulawesi. Organic materials of cow manure and chicken manure had fully been decomposed, whereas rice straw and soybean litter were agriculture wastes that had undergone the process of drying for two weeks after harvest.

Research Design

This study used a balanced randomized complete block design (RCBD) in a factorial pattern. The first factor was soybean variety, consisted of two levels ie: Argo Mulyo variety (V1) and Gema variety (V2); both varieties were not known of their resistance to bacterial pustules. The second factor was the application of Biofresh with various types of organic materials, consisted of five levels ie: Biofresh with cow manure (B1), Biofresh with chicken manure B2), Biofresh with rice straw (B3), Biofresh with soybean litter (B4), and Biofresh without organic matter (B5).

This experiment consisted of 10 treatment combinations with three replicates, so there were 30 experimental plots. In each plot, 10 diagonally random samples were selected, so overall there were 300 plant samples. Data were analyzed using a statistical analysis system version 9.1.3, followed by the Duncan Multiple Range Test at $\alpha = 0.05$.

Field Research Procedures

The land used for planting was cultivated with tractors, tillage was done twice, and 30 plots were made, each measuring 5 m x 6 m, with a height of 30 cm. The distance between plots in the same group was 30 cm, while among group of 50 cm.

Formulation of Biofresh

Biofertilizer "Biofresh" was made up of the mixture of rhizobacteria *Bacillus cereus* ST21b, *B. subtilis* ST21e, *Serratia* sp SS29a isolates and organic matters. A 100 ml of 48-h-old bacterial culture in their respective medium with a population of 10^9 to 10^{10} CFU/ml (OD_{550}= 0.5) was mixed with 1 kg of peat moss and sterile clay [9]. All manipulations were carried out under a sterile condition. Furthermore, the mixture of the three rhizobacteria and material formulation was dried for 48 h and packed in plastic bags and was ready for application.

Liming and organic matter application

Liming was done by evenly sowing Dolomite limestone above the surface of the plots with a dose of 1.3 tons/ha, two weeks before planting. Soybean litter waste and rice straw were in the form of dry chopped (± 5 cm) materials, applied as much as 3 tons/ha one week before planting by sowing evenly over the plots in accordance with corresponding treatments.

Planting

Soybean seeds were surface sterilized with 1.5% NaOCl and then rinsed three times with sterile water. Seeds were planted in the plots as deep as 3 cm, three seeds per hole. Planting spacing was 30 cm x 25 cm. Two weeks after planting, the plants were weeded and maintained two plants per hole.

Applications of bio-fertilizer "Biofresh" and inorganic fertilizer

Application of biofresh was performed twice on all treatments. The first application was at the time of planting, by making the biofresh as the cover of planting hole. The second application was done four weeks after planting by sprinkling near plant roots. The dose of each application was 10 g/plant.

In addition to providing biofertilizer, inorganic fertilizers were also used with a dose of 50% of the recommended dose, the best treatment of previous studies [11]. Urea fertilizer was given at two weeks and five weeks after planting, each by half of recommended dose. Phosphate (SP36) and Potassium (KCl) fertilizers were only given at two weeks after planting. The inorganic fertilization was done by dibble stick around the plants.

Observation variables

Disease Severity

Observations were made on 10 plant samples taken diagonally random on each plot. The assessment criteria were based on the numerical categories of attack, measured by the difference in the severity of pathogen infection on plants, namely: 0 = no attack , 1 = patches of pustules $\leq$ 5% of the leaf area, 2 = patches of pustules between 5 < x $\leq$ 15% of leaf area, 3 = patches of pustules between 15 < X $\leq$ 30% of the leaf area, 4 = patches of pustules between 30 < X $\leq$ 50% of the leaf area, 5 = patches of pustules > 50 [17]. Furthermore, the severity of the disease was calculated using the formula:

$$DS = \frac{\sum n_i v_i}{NZ} x100\%$$

Notes: DS = disease severity; ni = number of plants in each attack category, vi = the numerical value of each attack category, Z = the highest numerical value of attack category, and N = the number of plants observed

Area Under Disease Progress Curve (AUDPC)

The AUDPC value was calculated based on disease severity data to see the overall progression of the disease. The value was calculated based on the following formula [18].

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

Notes: yi+1 = the observation data at i+1; yi = the observation data at i, ti + 1 = observation time at i; ti = observation time of the first observation.

The AUDPC Value was used to calculate the disease suppression index. Disease suppression index is a number that can express the degree of effectiveness of a treatment against pathogens [19], calculated by the formula:

$$\text{IPP} = \frac{\text{Dic} - \text{Dib}}{\text{Dic}} \text{ x } 100\%$$

Notes: IPP (disease suppression index); Dic = AUDPC for control; Dib = AUDPC for treatment

Soybean growth and yield

Observed growth variables were number of leaves and plant height, measured at the ages of four and eight weeks after planting on 10 plant samples. Yield component variables were observed on the same 10 plant samples as for the above plant growth variables. The observed yield variables were the number of pods per plant, number of filled pods per plant, the number of seeds per plant, weight of 100 seeds per plant, seed weight per plant, and plant production (tons/ha)

RESULTS AND DISCUSSION

Disease severity: the effects of varieties and organic matters

Naturally occuring bacterial pustule disease began to appear at four weeks after planting (WAP). The research results showed that soybean variety significantly affected the development of the pustule disease; Argo Mulyo variety had better resistance than Gema variety. At 7 WAP, the disease severity on Gema variety reached 44.23%, while on Argo Mulyo variety was about 11.09% (Figure 1). The use of varieties that have high production ability and resistance to various external influences such as pathogen infections is desirable for the development of soybean in a wet tropical climate like in Indonesia.

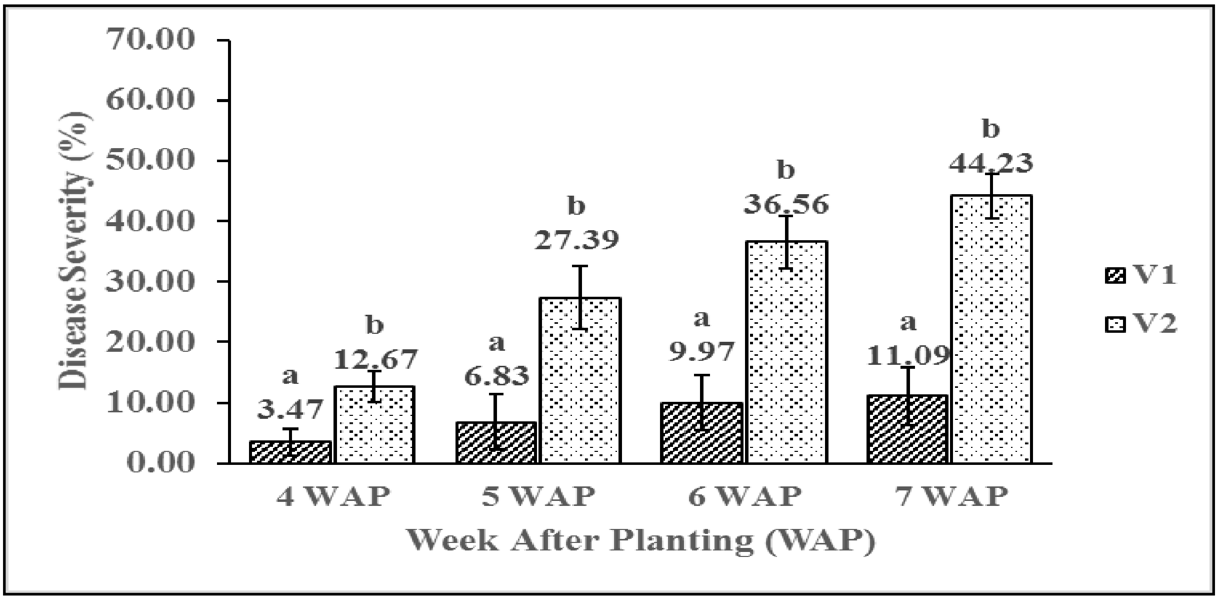

Figure 1: Severity of bacterial pustule disease on two different soybean varieties, V1: Agro Mulyo, V2: Gema

Organic matters significantly affected the development of soybean bacterial pustule disease. The effect of organic materials was more pronounced when they were combined with more appropriate varieties. The disease severity on Agro Mulyo variety applied with biofresh and soybean litter was always lower than that on the other treatments. The disease severity on that treatment at 7 WAP was only around 4.13%, while on the treatment without organic material, the severity was 14.93%. Disease severity on Gema variety was in the range of 40.33% to 48.80% (Table 1). The results of this study indicated that Argo Mulyo variety was more resistant to bacterial pustule disease than Gema variety. It had suggested that a single factor variety turned out to give a significant effect on the development of bacterial pustule disease in the field [4].

Table (1): Effect of treatment combinations of Biofresh and various organic materials in two soybean varieties on pustule disease severity

Treatment combination	Disease severity (%) at observation			
	4 WAP	5 WAP	6 WAP	7 WAP
Argo Mulyo + cow manure (V1B1)	1,86 d	4,00 cd	5,87 d	8,26 c
Argo Mulyo + chicken manure (V1B2)	5,06 c	8,00 c	13,33 c	14,00 bc
Argo Mulyo + rice straw (V1B3)	2,66 d	4,80 cd	10,53 cd	14,13 bc
Argo Mulyo + soybean litter (V1B4)	1,33 d	3,20 d	4,08 d	4,13 d
Argo Mulyo + without organic material (V1B5)	6,40 c	14,13 b	14,33 c	14,93 b
Gema + cow manure (V2B1)	14,53 a	29,73 a	34,27 b	40,33 a
Gema + chicken manure (V2B2)	13,86 a	29,33 a	38,13ab	46,40 a
Gema + rice straw (V2B3)	10,40 b	28,53 a	35,93 b	45,07 a
Gema + soybean litter (V2B4)	9,33 b	18,13 b	31,53 b	40,53 a
Gema + without organic material (V2B5)	15,20 a	31,20 a	42,93 a	48,80 a

Notes: The numbers followed by the same letters in the same column do not significantly different at 95% confidence level.

The lowest disease progression was on treatment plots applied with biofresh combined with soybean litter. At the end of the observations (7 WAP), disease severity for the treatment of biofresh with organic matter soybean litter was 22.33%, significantly different from those of other treatments except with that of the treatment of Biofresh with cow manure, in which the severity of the disease was 24.30%. Disease severity on treatment without organic material was 31.90% (Figure 2). Application of soybean litter had a significant effect in improving the effectiveness of the biological fertilizer biofresh in inhibiting the progression of bacterial pustule disease on Argo Mulyo and Gema varieties.

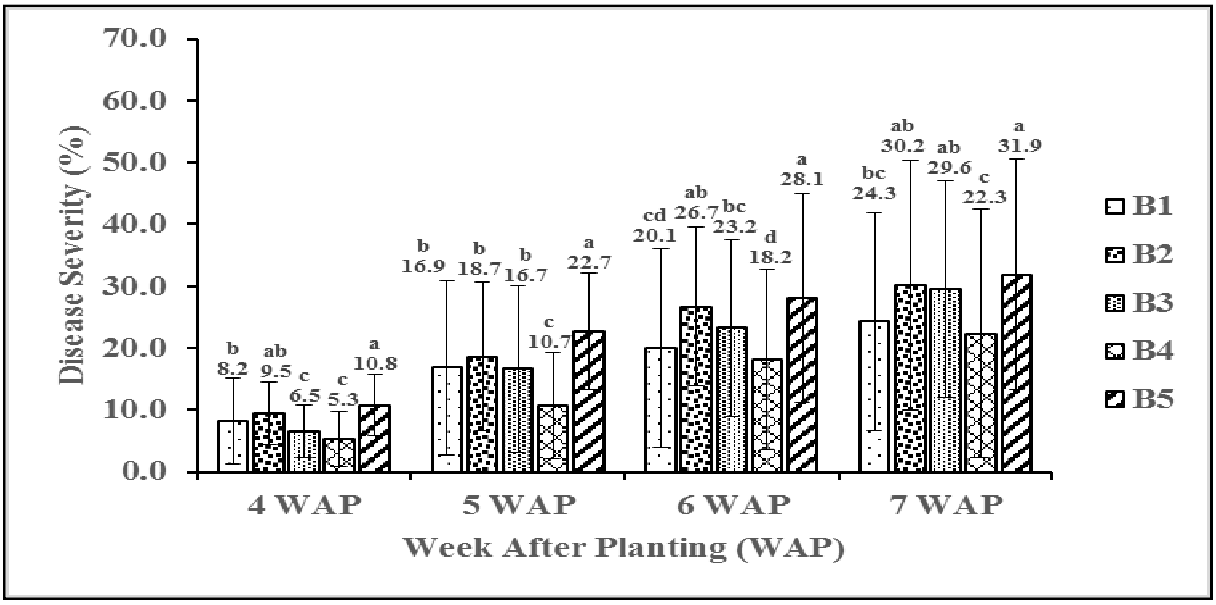

Figure 2: The severity of the bacterial pustule disease on soybean applied with Biofresh treatment with different organic materials. B1: cow manure; B2: chicken manure; B3: rice straw; B4: soybean litter waste, and B5: no organic material (control)

Plant resistance can be [20]: (1) genetic, resistant properties are governed by the genetic trait that can be inherited, (2) morphology, resistant properties due to the nature of plant morphology unfavorable to pathogens, (3) chemical, resistance caused by chemicals produced by plants. In addition, the plant resistance to pathogens can be formed in the presence of resistance mechanisms affected as a result of the introduction of the biotic elicitor in the form of biological agents that are able to induce plant resistance.

It was stated by [21] that the content of salicylic acid on the sprouts of tobacco plants (*Nicotiana tabacum*) treated with three strains of PGPR increased significantly in the first week after PGPR treatment. Similarly, it was mentioned [22] that increased corn plant resistance to downy mildew induced by elicitor biotic rizobacteri was associated with an increase in salicylic acid content in the leaf tissue of maize plants tested. Plant varieties resistant to the bacterial pustule disease suspected to produce root exudates with compounds needed by microorganisms [4]. This supports the statement that the relationship between plants and soil microorganisms can be compatible if compounds and organic acids released by the plant suited to the needs of these microorganisms, so they can grow optimally and mutualistic symbiosis with plants [23].

Area Under Disease Progress Curve (AUDPC)

Analysis of pustule disease progression on soybean plants was done by calculating the AUDPC which can be seen in Figure 3. Overall AUDPC values and the effectiveness of each treatment in reducing AUDPC can be seen in Table 2. Data in Table 2 show that in general the treatment combination of Argo Mulyo variety with different types of organic materials had lowest AUDPC values compared to the treatment combination of Gema variety with different types of organic materials.

AUDPC observations showed that the combination treatment of biofresh and soybean litter on Argo Mulyo variety gave the lowest AUDPC value of 75.1% week, while on the treatment without organic matter with the same variety, the AUDPC value amounted to 266.9% week. This indicated that the soybean litter can increase the effectiveness of the biofresh to 71.9% in inducing plant resistance to bacterial pustule disease on Argo Mulyo variety, while on Gema variety was only 29.7%.

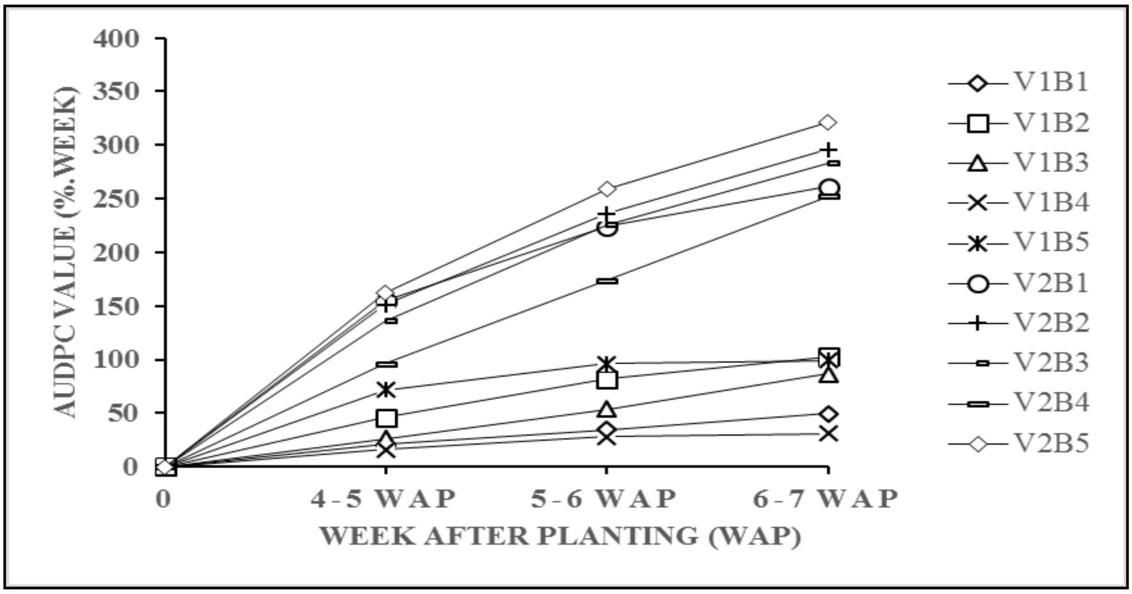

Figure 3: AUDPC value of bacterial pustule disease on two soybean varieties, applied with a combination treatment of biofresh and various organic materials. V1 = Argo Mulyo variety; V2: Gema variety; B1 = cow manure; B2 = chicken manure; B3 = rice straw; B4 = soybean litter, B5 = without organic material

Table (2): Effect of various organic materials on the effectiveness of biofresh in increasing the number of leaves and plant height of soybean crop in sub-optimal Ultisol land

Treatment combination	Leaf number		Plant height (cm)	
	4 WAP	8 WAP	4 WAP	8 WAP
Argo Mulyo + cow manure (V1B1)	3,56 cd	13,17 c	11.41 ab	23.41 c
Argo Mulyo + chicken manure (V1B2)	3,70 ab	13,90 c	12.24 ab	27.26 bc
Argo Mulyo + rice straw (V1B3)	4,10 ab	13,83 c	12.13 ab	23.12 c
Argo Mulyo + soybean litter (V1B4)	4,30 a	16,43 bc	13.60 ab	24.47 c
Argo Mulyo + without organic material (V1B5)	3,30 cd	11,00 d	10.75 b	16.25 d
Gema + cow manure (V2B1)	3,13 d	13,70 c	15.13 a	31.32 ab
Gema + chicken manure (V2B2)	3,30 cd	16,73 b	12.20 ab	32.99 ab
Gema + rice straw (V2B3)	3,50 cd	13,83 c	12.34 ab	33.97 ab
Gema + soybean litter (V2B4)	3,66 cd	19,86 a	13.57 ab	34.97 a
Gema + without organic material (V2B5)	3,16 cd	15,15 bc	12,14 ab	33,99 ab

Notes: The numbers followed by the same letters in the same column do not significantly different at 95% confidence level. WAP = week after planting.

Soybean growth and yield

Research results showed that treatment of soybean litter on Gema variety provided the highest number of leaves and plant height at the end of the observation (8 WAP), which were 19.86 trifoliate leaves and plant height of 34.97 cm (Table 3). Gema variety was also better in the number of pods and pod dry weight, while Agro Mulyo variety was better in seed weight. While on treatments of organic materials independently, soybean litter always gave higher yields, better on number of pods, pod dry weight, and seed weight (Figure 4).

Application of biofresh in the combination treatment of Argo Mulyo variety and soybean litter can increase resistance and soybean production when compared with other treatments. The role of organic matter soybean litter and application of biofresh allegedly gave effect on Argo Mulyo variety to increase production and plant resistance, so the variety was capable of producing 1.54 tons/ha and reached the minimum production potential of 1.5 to 2.0 tons/ha, although planted at the peak of the dry season. This indicates that the organic material played a role in biofresh effectiveness in increasing crop production by 77.1% compared to the treatment without any organic material (Figure 4). Similar results on the effects of bio-fertilizers on flax productivity and quality have previously been reported [24].

Table (3): Total AUDPC value and effectiveness of biofresh with various types of organic material to suppress the severity of soybean pustule disease

Treatment	AUDPC Value (% per week)	Effectiveness to suppress AUDPC (%)
Argo Mulyo + cow manure (V1B1)	104,13	60,8
Argo Mulyo + chicken manure (V1B2)	230,07	37,8
Argo Mulyo + rice straw (V1B3)	166,13	13,8
Argo Mulyo + soybean litter (V1B4)	75,13	71,9
Argo Mulyo + without organic material (V1B5)	266,93	0
Gema + cow manure (V2B1)	640,03	13,9
Gema + chicken manure (V2B2)	683,20	8,1
Gema + rice straw (V2B3)	645,40	13,1
Gema + soybean litter (V2B4)	522,20	29,7
Gema + without organic material (V2B5)	742,93	0

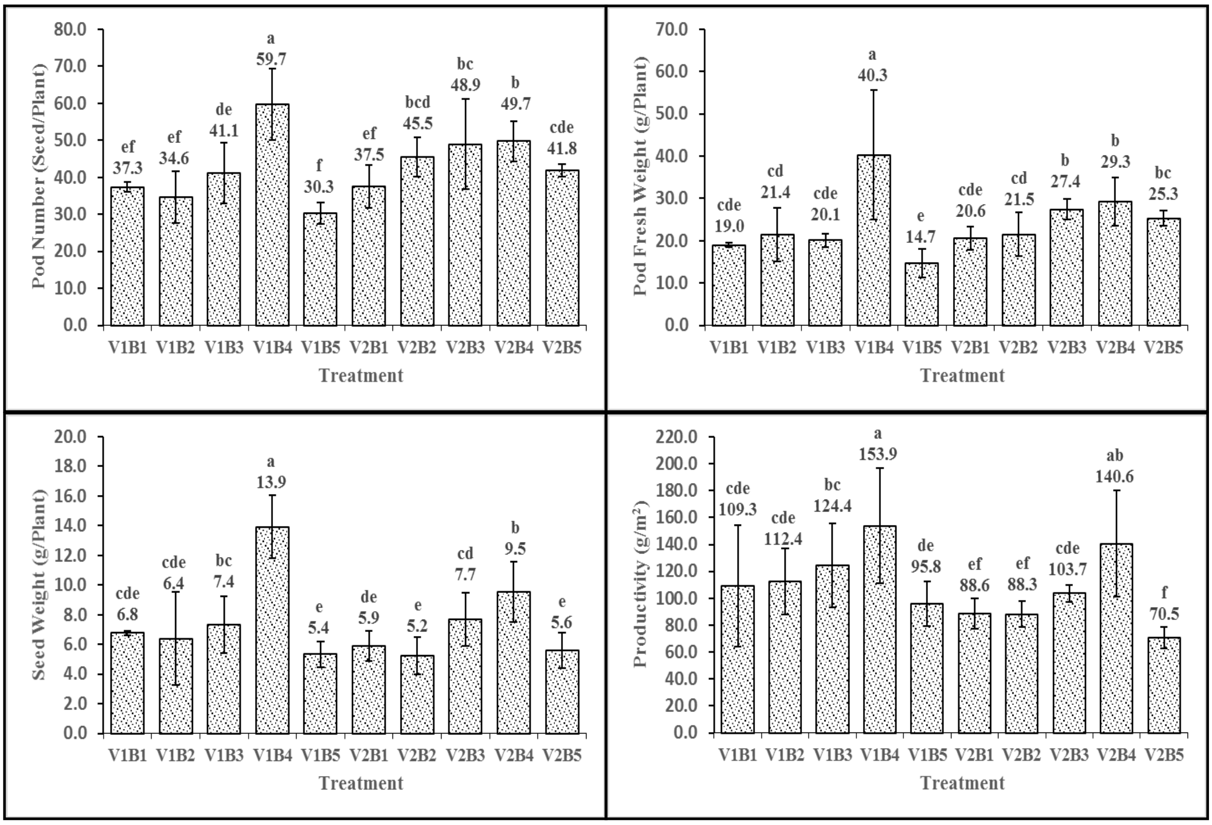

Figure 4: Effect of various organic materials on the effectiveness of biofresh in increasing soybean yields on marginal Ultisol land. The numbers followed by the same letter on the same figure, do not significantly different, at the confidence level of 95%.

Application of organic material was capable of enhancing the role of microbial constituents of biofresh i.e. *Bacillus subtilis* ST21e, *Bacillus cereus* 21b and *Serratia* sp SS29a. Higher inputs of organic matter provided energetic substrate for the present microbial communities that were activated to assure the turnover of applied nutrients [25]. The organic matter in organic farming systems can improve soil microbial biomass by 100% to 300% compared to the conventional agricultural system without the input of organic material [26]. It was suggested that the addition of organic matter to the soil will increase the activity of microorganisms as decomposer of organic material [27].

The content of the C/N ratio affects the rate of decomposition of organic material by microbial decomposer. This is in line with the statement that the legume was better used as an organic material because it has low C/N ratio when compared to non-legume crops with much higher C/N ratio [28]. High C/N ratio can lead to longer decomposition and slower process of nutrient mineralization of legume crops [29]. Thus the microbes contained in the biological fertilizer biofresh allegedly faster decomposing organic material legume litter so that more quickly available to plants. In addition, soybean litter becomes nutrients for microbial constituent of biofresh so as to increase its activity as a biological agent in colonizing plant roots.

Bacterial pustule infections have contributed to a decrease in the production of soybeans. In this study, although Gema variety genetically has higher production potential than Argo Mulyo variety, but because of the higher severity of the disease on Gema variety then its production was lower compared with the production of Argo Mulyo.

CONCLUSION

Organic matters improve Biofresh effectiveness in increasing soybean health and yield. Soybean variety also influences disease development. Soybean litter in combination with biofresh allegedly gave better effect on Argo Mulyo variety in increasing plant production, which reached 1.54 tons/ha, and increase of over 77 % compared to treatments without organic material. The biofertilizer Biofresh in combination with organic matter, especially soybean litter, can then be applied to reduce the severity of soybean pustule disease and increase soybean production.

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REFERENCES

[1] Sinclair JB. 1999. Bacterial pustule. In: Hartman G.L., Sinclair J.B., Rupe J.C. (eds): Compendium of soybean disease, 4th Ed. St. Paul Minnesota (US), APS.
[2] Khaeruni A, Suwanto A, Tjahjono B, Sinaga SM. 2007. Quick detection of soybean bacterial pustule disease using a PCR technique with specific primers. Hayati Journal of Biosciences 14: 76-80.
[3] Dirmawati SR. 2004. Study on the effectiveness of several components of environmentally friendly control methods against soybean bacterial pustule disease [PhD Desertation] Postgraduate School, Bogor Institute of Agriculture, Indonesia.
[4] Widjayanti T. 2012. The use of rice straw mulch and Plant Growth Promoting Rhizobacteria to suppress bacterial pustule disease of soybean. Jurnal Fitopatologi 8 (6): 161-169.
[5] Zehnder GW, Murphy JF, Sikora EJ, Klopper JW. 2001. Application of rhizobacteria for induced resistance. European Journal of Plant Pathology 107: 39-50.
[6] Salaheddin K, Valluvaparidasan V, Ladhalakshmi D, Velazhahan R. 2010. Management of bacterial blight of cotton using a mixture of *Pseudomonas fluorescens* and *Bacillus subtilis.* Plant Protection Science 46: 41-50.
[7] Javandira C, Aini LQ, Sugiharto AN, Abadi AL. 2013. The Potency of *Bacillus* sp. and *Pseudomonas* sp. as biological control agents against corn leaf blight disease caused by P*antoea* sp. J. Agrivita 35: 103-109.
[8] Khaeruni A, Wijayanto T, Syair. 2013. The utilization of indigenous rhizobacteria as an inducer for rice plant resistance against bacterial leaf blight disease (*Xanthomonas oryzae* pv. *oryzae*). In: Proceeding the 8th International Conference on Innovation and Collaboration Towards Asean Community 2015, Kendari, Indonesia.
[9] Khaeruni A, Rahim A, Syair, Adriani. 2014. Induction of resistance to bacterial leaf blight disease of rice using indigeneous rhizobacteria. Jurnal Hama dan Penyakit Tumbuhan Tropika 14: 57-63.

[10] Putra C, Giyanto. 2014. Compatibility of *Bacillus* spp. and Actynomicetes as biological agent for *Xanthomonas oryzae* pv. *oryzae* and rice growth inducer. Jurnal Fitopatologi Indonesia. 10: 160-168.
[11] Khaeruni A, Wijayanto T, Sutariati GAK, Asniah, Sulqifly. 2015. Improvement of resistance against pathogens, growth, and yield of soybean on marginal land using indigenous rhizobacteria formulations. In: Proceedings of the 17th International Conference on Mathematical and Computational Methods in Science and Engineering (MACMESE '15). Recent Advances in Mathematical and Computational Methods, Kuala Lumpur, April 23-25, 2015.
[12] Khaeruni A, Sutariati GAK, Wahyuni S. 2010. Characterisation and test of rhizosphere bacterial activity on ultisols as growth inducer and *in-vitro* biological agents of soilborne fungal pathogens. Jurnal Hama dan Penyakit Tumbuhan Tropika 10: 123-130.
[13] Satrah, VN. 2014. Stability of indigeneous rhizobacteria at type and material formulations and its role in improving resistance and production of soybean (*Glycine max*L. Merill) on Ultisols [Master Thesis], Prograduate Program, University of Halu Oleo, Kendari, Indonesia.
[14] Wang F, Tong YA, Zhang JS, Gao PC, Coffie JN. 2013. Effects of various organic materials on soil aggregate stability and soil microbiological properties on the Loess Plateau of China. Plant Soil Environment 59: 162–168.
[15] Acquaah G. 2005. Principles of Crop Production. Theory, Technique, and Technology. Pearson, Prentice Hall, New Jersey.
[16] Costal M, Beltaro J, Brito JCD, Neves MA, Guerrero C. 2010. The sludge and manure effects on the growth of citrus trees. WSEAS Transactions on Environment and Development 11: 721-730.
[17] Khaeruni A, Tjahjono B, Suwanto A, Sinaga MS. 2006. Virulence of several *Xanthomonas axonopodis* pv *glycines* isolates from Edamame to three soybean varieties. Jurnal Hama dan Penyakit Tumbuhan Tropika 8: 39-45.
[18] Van Der Plank JE. 1963. Plant Disease: Epidemics and Control. London (UK) Academic Press.
[19] Nawangsih AA, Wardani FF. 2014. Interaction between endofitic bacteria and rhizosphere bacteria in suppressing bacterial wilt disease of tomato. Jurnal Fitopatologi Indonesia. 10: 145-152.
[20] Agrios GN. 2005. Plant Pathology. 5th Ed. Amsterdam-Boston, Elsevier-Academic Press.
[21] Zhang Y. 2004. Biocontrol of sclerotinia stem rot of canola by bacterial antagonists and study of biocontrol mechanisms involved. [Master Thesis]. Winnipeg, Canada, Departement of Plant Science, University of Manitoba.
[22] Hoerussalam, Purwantoro A, Khaeruni A. 2013. Induction of maize (*Zea mays*L.) resistance to "bulai" disease through seed treatment and its genetic transmission to S1 generation. Jurnal Ilmu Pertanian 16: 42-59.
[23] Glick BR, Patten CL, Holguin G, Penrose DM. 1999. Biochemical and Genetic Mechanism Used by Plant Growth Promoting Bacteria. Ontario (CA), Imperial Collage Press.
[24] Bakry AB, Sadak MSh, Abd Allah MMS, El-Razik TMA, and Dawood MG. 2016. Maximizing the performance, productivity and quality traits of two flax cultivars by using some bio-fertilizers under newly reclaimed sandy soil. Res. J. of Pharm. Biol. and Chem. Sci. 7(6): 429-441.
[25] Araujo ASF, Leite LFC, Santos VB, Carneiro RFV. 2009. Soil microbial activity in conventional and organic agricultural systems. Sustainability 1: 268-276.
[26] Santos VB, Araujo AS, Nunes LAPL, Melo WJ. 2012. Soil microbial biomass and organic matter fractions during transition from conventional to organic farming systems. Geoderma 170: 227–231.
[27] Wijanarko A, Purwanto BH, Shiddieq D, Indradewa D. 2012. The effect of organic matter quality and soil fertility on nitrogen mineralization and absorption of cassava plants on Ultisol. Jurnal Perkebunan & Lahan Tropika 2: 1-14.
[28] Isrun. 2010. The changes of nitrogen absorption on maize and Al-exchange capacity as a result of the application of legume and non-legume composts on inseptisols Napu. Jurnal Agroland 17: 23-29.
[29] El-Gamal NG, Atalla SMM, and El-Shammy AR. 2017. Studies on the effect of olive mill waste on pre-harvest and plant fungal diseases. Res. J. of Pharm. Biol. and Chem. Sci. 8(1): 1730-1738