Effectiveness of the composition of bokashi organic materials on the ability of biofresh biological agents in inducing resistance of three maize varieties to sheath blight disease (*Rhizoctonia solani*)

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Abstract. The limited availability of fertile land and the existence of disease-causing pathogens are major obstacles to the development of maize plants. This study aimed to get at the effect of the composition of organic matters on the ability of Biofresh biological agents in inducing the resistance of three maize varieties to sheath blight disease. The study used a factorial Randomized Block Design. The first factor consisted of three levels, namely: V₁ = Hybrid Variety BISI-2, V₂ = Variety Pioneer-35, and V₃ = Variety Pertiwi-3. The second factor consisted of three levels, namely: B₀ = 100% inorganic fertilizer, B₁ = Biofresh + bokashi + 50% inorganic fertilizer, B₂ = Biofresh + Bokashi Plus + 50% inorganic fertilizer. The highest disease severity occurred in the independent treatments of V₂ variety (38.19%), and B₀ (49.25%), the highest AUDPC value occurred at treatment V₂B₀ (513.33% unit), the highest IPP was obtained at V₃B₂ treatment (52.78%), the highest increase in salicylic acid activity and peroxidase enzyme activity, from the vegetative phase to the generative phase, was found at V₃B₂ treatment, each at 1.34 and 1.21, respectively.

1. Introduction

Maize (*Zea mays*) is one of the cereal plants that have significant economic value. The need for maize is increasing along with market demand because maize can be used as food, feed, and industrial materials. Southeast Sulawesi is one of the areas that has potential land for the development of maize, but the availability of fertile land in this area is quite limited because 60.3% of the land is dominated by marginal land Ultisol [1]. Ultisol is a soil that has soil acidity problems, low organic matter and low macro nutrition, and has deficient P availability [2]. In addition to the availability of limited fertile land, the presence of pathogens that because disease is also an obstacle to the development of maize plants. A critical disease in maize plants is sheath blight caused by *Rhizoctonia solani* [3]. Mulyati [3] further stated that in high attacks, this disease could result in a 100% loss of maize yield. The
existence of this disease has been widely reported to spread in several maize production centers. In Asia, such as China and India, this disease results in up to 100% loss [4, 5], and in the United States, the distribution of sheath blight is also quite extensive [6]. Therefore, it is essential to make efforts to increase the carrying capacity of Ultisol soil and the application of disease control techniques in order to increase maize productivity in Indonesia, especially in Southeast Sulawesi.

To overcome this problem, we need an appropriate technology that has ecological insight. The application of environmentally friendly technology to increase the carrying capacity of marginal Ultisol land, namely through the provision of organic materials, liming, selection of adaptive varieties and high production, and the use of biological agents became one of the essential efforts in increasing maize production. The development of local microbial-based cultivation technology to improve adaptability, disease resistance, and productivity of maize crops on marginal land of ultisol has so far shown a significant influence. One of the local microbial products that has been developed is “Biofresh” biological fertilizer. Biofresh biological fertilizer contains three types of local rhizobacteria, *Bacillus subtilis* ST21e, *B. cereus* ST21b, and *Serratia* sp. SS29a. The three rhizobacterial strains have the ability to produce IAA dissolve phosphate and fixate N from the air and are able to produce degrading enzymes such as proteinase, chitinase and cellulase which can suppress the development of Rhizoctonia and Sclerotium wilt in soybean and maize plants and can stimulate second vegetative growth these plants [7] and were able to reduce the use of N, P, K fungicides and inorganic fertilizers by 40-60% in maize, tomato, and maize crops on a greenhouse scale [8]. The use of local microbes will be more effective when combined with the use of organic materials. Organic material is also an energy source for the life of soil organisms that carry out various essential processes in the soil [9, 10].

This study aimed to get the effect of the composition of bokashi organic matter on the ability of Biofresh biological agents to induce the resistance of three maize varieties to sheath blight disease.

2. Materials and methods

2.1. Materials

The materials used were organic fertilizer Bokashi and Bokashi Plus, Biofresh biological fertilizer, maize seeds BISI 2 variety, Pertiwi 35 variety, Pioner 3 variety, decomposers (bio activators), inorganic fertilizers (Urea, SP36 and KCl), and dolomite lime (CaMg (CO₃)₂).

2.2. Methods

This study used a factorial Randomized Block Design. The treatments tested were: Varieties, consisting of three treatment levels (V1 = BISI-2 Hybrid Variety, V2 = Pioneer-35 Variety, and V3 = Pertiwi-3 Variety); and Organic matter, consisting of three treatment levels (B0 = 100% inorganic, B1 = Biofresh + Bokashi + 50% inorganic fertilizer, B2 = Biofresh + Bokashi Plus + 50% inorganic fertilizer). Each treatment was repeated 3 times.

2.3. Biofresh, biofresh plus and inorganic chemical fertilizer applications

Biofresh application on the Biofresh + bokashi (B1) treatment plot was carried out twice, the first application was carried out during planting which was used as seed cover in planting holes with a dose of 10 g / hole and the second application was carried out at 4 WAP week after planting (WAP) by spreading around the stem and roots plants with a dose of 24 g / clump. The Biofresh + Bokashi Plus (B2) treatment is applied only once as a seed cover in the planting hole. The application of inorganic fertilizers in the control treatment (B0) was at a dose of 100% of the recommended dosage, while in treatments B1 and B2 used only 50% of the recommended dosage of inorganic fertilizers.

2.4. Observed variables

2.4.1. Severity of sheath blight disease. Disease Severity (DS) was calculated based on the formula:
\[ DS = \frac{\sum (n_i \cdot v_i)}{N \cdot Z} \times 100\% \] (1)

Note: \( n \): number of leaves/sheaths attacked in each category; \( v \): score on each leaf/sheath attacked; \( Z \): highest score; \( N \): number of leaves/sheath observed.

The value of banded leaf and sheath blight disease in maize plants was determined according to the procedures of Ahuja and Payak [11], in which Score 1: symptoms in only 1 of the lowest leaf sheaths with very small and little lesion; Score 2: symptoms in the lower two sheaths with dilated lesion; Score 3: the symptoms have arrived at the fourth leaf sheaths from the bottom, many lesions and fused; Score 4: equal to scale 3, only color changes occur with small lesions; Score 5: symptoms in all sheaths, except 2 segments below the cob; Score 6: symptoms of the disease have arrived at the place where the cob is attached, but the cob has not been infected; Score 8: the symptoms of the disease have reached the cob and the leaf surface turns white like a ribbon, the size of the cob is not normal and some plants are dead; Score 9: like a score of 8, the stem shrinks, the shape of the cob is not normal, and the arrangement of seeds is irregular; generally the plants die prematurely and sclerotium is often found on the cob and hair.

The results of the calculation of disease severity were used to calculate the Area Under Disease Progress Curve (AUDPC) value, which is to determine the progress of the disease as a whole, based on the following formula of Van der Plank [12]:

\[ \text{AUDPC} = \sum \frac{X_i + X_{i+1}}{2} (t_{i+1} - t_i) \] (2)

Where; \( X_i \): First observation data, \( X_{i+1} = 1\) st observation data + 1, \( t_i \): 1st observation time, \( t_{i+1} = 1\) st observation time + 1.

2.4.2. The disease suppression index (DSI). DSI was calculated based on the AUDPC value. DSI is the level of effectiveness (affordability) of controlling a biocontrol agent against pathogens, calculated by formula, according to Nawangsih et al. [13].

\[ \text{DSI} = \frac{D_{ic} - D_{ib}}{D_{ic}} \times 100\% \] (3)

Where; DSI = Disease Suppression Index, \( D_{ic} \) = AUDPC on control, \( D_{ib} \) = AUDPC on Biofresh treatment.

2.4.3. Crop resistance analysis. Analysis of maize crop resistance to disease was carried out based on the production of Salicylic acid and the Peroxidase enzyme activity, in the vegetative phase (4 WAP) and generative phase (8 WAP).

2.4.3.1. Salicylic acid. Analysis of the content of salicylic acid was carried out using the method proposed by Warrier et al. [14]. Measurement of salicylic acid content used UV-Vis spectrophotometer, test mixture solution (9 mL) containing 1 mL extract of plant leaves (results of 1:10 g /v leaf samples) and 8 mL FeCl3. Leaf samples were cut into small pieces, and as much as 1 g was put into Erlenmeyer, a 50 mL 50% ethanol solution was added and then shaken for 15 minutes at 150 rpm, then filtered. The filtrate was put into Eppendorf and centrifuged for 10 minutes at a speed of 12,000 rpm with a temperature of 250C and a 1% FeCl3 solution was added. Absorbance was measured at a wavelength of 525 nm.

2.4.3.2. Peroxidase enzyme. Analysis of peroxidase enzyme activity was carried out using the method of Petra and Mishra [15]. Measurement of peroxidase enzyme activity used UV-Vis
spectrophotometer, test mixture solution (7 mL) containing 1 mL phosphate buffer (pH 6.5), 3 mL pyrogallol, 2 mL 1% H₂O₂ and 1 mL plant leaf extract (result of leaf sample 1:10 grinding) g/v). Absorbance was measured at 420 nm wavelength, the amount of purpurogalin was formed for about 5 minutes.

3. Results and discussion

3.1. The severity of the disease
The composition of organic matter affected suppressing the severity of banded leaf and sheath blight at 4 WAP, 6 WAP, 8 WAP and 10 WAP. Independent treatment of organic matter had a significant effect on the severity of sheath leaf blight at 4, 6 WAP, 8 WAP and 10 WAP (Table 1), while the effect of independent variety showed a significant effect on 10 WAP (Table 2).

Table 1. The independent effect of organic matter on the severity of sheath blight disease

| Treatment | 4 WAP | 6 WAP | 8 WAP | 10 WAP |
|-----------|-------|-------|-------|--------|
| B0        | 9.26a | 13.70a| 20.86a| 49.25a |
| B1        | 5.43b | 8.64b | 16.30b| 29.87b |
| B2        | 3.94c | 5.80c | 11.97c| 23.08c |

Note: The numbers in the same column followed by the same letters are not significantly different according to the DMRT test at the 95% confidence level. WAP: Week After Planting.

The results of the study (Table 1) showed that the independent treatment of organic matter had a significant effect on the severity of banded leaf and sheath blight disease. The highest disease severity was shown in independent B0 treatment at 4 WAP (9.26%), 6 WAP (13.70%), 8 WAP (20.86%) and 10 WAP (49.25%), which were significantly different from those of independent treatments B1 and B2. Consistently the B2 treatment was the best (lowest disease severity) at 4 WAP (3.94%), 6 WAP (5.80%), 8 WAP (11.97%), and 10 WAP 23.08%.

Table 2. The independent effect of variety on the severity of sheath blight disease

| Treatment | 4 WAP | 6 WAP | 8 WAP | 10 WAP |
|-----------|-------|-------|-------|--------|
| V1        | 6.54tn| 9.26tn| 17.03tn| 34.14b |
| V2        | 6.42tn| 9.01tn| 15.92tn| 38.19a |
| V3        | 5.56tn| 9.83tn| 16.17tn| 29.87c |

Note: The numbers in the same column followed by the same letters are not significantly different according to the DMRT test at the 95% confidence level. WAP: Week After Planting.

The results of the study (Table 2) showed that the independent treatment of variety had a significant effect on the severity of midrib blight disease at 10 WAP. The severity of the disease among varieties (V1, V2 and V3) was not significantly different at 4, 6 and 8 WAP. However, at 10 WAP, treatment V3 gave the lowest disease severity of 29.87%, significantly different from those of treatments V1 and V2 with 34.14%, and 38.19% disease severity, respectively.

3.2. Area under the disease development curve (AUDPC) and disease suppression index (DSI)
The results of the average AUDPC and DSI analysis (Table 3) show that the application of organic matter greatly influenced Biofresh biological agents’ ability to suppress the development of sheath blight disease. At the age of 56-70 days, the lowest AUDPC value was shown in the combination of treatment V3B2 with AUDPC value of 207.41% unit, while the highest AUDPC value was obtained in combination treatment V2B0 with AUDPC value of 513.33% unit. In addition to influencing the
ability of biological agents to suppress disease progression, the composition of organic matter influenced the ability of Biofresh biological agents to improve the Disease Suppression Index (DSI). The highest Disease Suppression Index was shown in V3B2 treatment with DSI value of 52.78%.

Table 3. The independent effect of variety on the severity of sheath blight disease

| Treatment | AUDPC Value of and Sheath Blight Disease unit) | DSI (%) |
|-----------|-----------------------------------------------|---------|
|           | Day After Planting                            |         |
|           | 28-42                                        | 42-56   | 56-70   |         |
| V1B0      | 171.11                                       | 248.89  | 487.41  | 0       |
| V1B1      | 93.33                                        | 171.11  | 331.85  | 36.21   |
| V1B2      | 67.41                                        | 132.22  | 256.67  | 51.61   |
| V2B0      | 147.78                                       | 235.93  | 513.33  | 0       |
| V2B1      | 108.89                                       | 178.89  | 350.00  | 27.44   |
| V2B2      | 67.41                                        | 108.89  | 272.22  | 51.73   |
| V3B0      | 163.33                                       | 241.11  | 471.85  | 0       |
| V3B1      | 93.33                                        | 173.70  | 287.78  | 36.61   |
| V3B2      | 70.00                                        | 132.22  | 207.41  | 52.78   |

3.3. The activity of salicylic acid and peroxidase enzyme

The composition of organic matter affected the ability of Biofresh biological agents to increase the activity of salicylic acid and peroxidase enzymes in maize plants. In general, there was an increase in the activity of salicylic acid and peroxidase enzyme activity in all treatments but with different levels. The highest activity of salicylic acid and peroxidase enzyme occurred in the generative phase of V3B2 treatment, each at 1.90 and 1.80, with an increase in salicylic acid levels of 1.34, and an increase in peroxidase enzyme activity of 1.21 (Table 4).

Table 4. The activity of salicylic acid and peroxidase enzyme

| Treatment | Activity of Salicylic Acid | Activity of Peroxidase Enzyme |
|-----------|----------------------------|-------------------------------|
|           | Vegetative Phase | Generative Phase | Increase | Vegetative Phase | Generative Phase | Increase |
| V1B0      | 0.04            | 0.09               | 0.05     | 0.18            | 0.26               | 0.08     |
| V1B1      | 0.20            | 1.09               | 0.89     | 0.23            | 0.79               | 0.56     |
| V1B2      | 0.35            | 1.17               | 0.82     | 0.39            | 1.08               | 0.69     |
| V2B0      | 0.12            | 0.14               | 0.02     | 0.22            | 0.89               | 0.67     |
| V2B1      | 0.45            | 1.28               | 0.83     | 0.26            | 1.17               | 0.91     |
| V2B2      | 0.46            | 1.22               | 0.76     | 0.27            | 1.25               | 0.98     |
| V3B0      | 0.14            | 0.35               | 0.21     | 0.18            | 1.07               | 0.89     |
| V3B1      | 0.48            | 1.29               | 0.81     | 0.37            | 1.32               | 0.95     |
| V3B2      | 0.56            | 1.90               | 1.34     | 0.59            | 1.80               | 1.21     |

4. Discussion

The results showed that the treatment had a significant effect on disease severity, area under the disease development curve (AUDPC), disease suppression index (DSI) and stimulated the activity of salicylic acid and peroxidase enzyme activity. Furthermore, the results of this study also showed that the independent effects of organic material (B) and variety (V) significantly determined the ability of
Biofresh biological agents to induce disease resistance in maize plants. It can be seen in the observation of the severity of the disease where the treatment of independent B2 treatment gave the lowest disease severity of 23.08%, far lower than the severity of the disease in the control treatment (B0), which was 49.52%. In the independent treatment of variety, the lowest disease severity value occurred at V3 of 29.87%. The disease severity value was directly proportional to the AUDPC value, where treatment V2B0 showed the highest AUDPC value that was equal to 513.33 unit. The AUDPC value also affected the DSI value, where the highest DSI value occurred in the V3B2 treatment with a DSI value of 52.78%.

The composition of organic matter and the use of variety played a significant role in Biofresh biological agent's ability to reduce the severity of midrib blight disease. It is in line with the research of Widjayanti [16], Nguyen et al. [17] and Sulianti et al. [18], where variety had a significant influence on reducing the severity of bacterial diseases in the field. In the treatment of independent B2 treatment (Biofresh + Bokashi Plus biological agent), the organic matter content was more complex than in treatment B1. In B2 treatment, organic materials that made up Bokashi Plus consisting of soybean litter, rice straw, cow manure, chicken manure, and rice bran were thought to be more stable, which greatly influenced the performance of Biofresh biological agents in inducing maize plant resistance. It is in line with the research of Wijayanto et al. [19] who reported that the use of organic matter affected Biofresh's ability to improve the health and yield of soybean crops.

The ability of biological agents to induce the resistance of maize plants against *Rhizoctonia solani* can be seen from the increased activity of salicylic acid and the activity of the peroxidase enzyme. The treatment of V3B2 gave an increase (from the vegetative phase of the plant to the generative phase) in the activity of salicylic acid and the highest peroxidase enzyme activity, i.e., 1.34 and 1.21. Biofresh biological agent consists of three microorganisms, namely *Bacillus subtilis* ST21e, *B. cereus* ST21b, and *Serratia sp.* SS29a could secrete extracellular enzymes (chitinase, cellulose, and proteinase) [8]. This further proved Biofresh biological agent's ability to induce the resistance of maize plants against the pathogen *R. solani*. The activity of salicylic acid and peroxidase enzyme activity was related to plant resistance reactions. Murphy et al. [20] reported that salicylic acid was signal transduction in which one branch activates PR-proteins, including peroxidase. Vlot et al. [21] reported that salicylic acid activity in plants was found and shown to be involved in plant defenses against pathogenic infections. The most essential role known was as a signal in the activation of the defense response to pathogens. It is also in line with the results of the study of Fragniere et al. [22], which showed that salicylic acid was induced after pathogenic infection or stress due to biotic and abiotic factors. The peroxidase enzyme acts as a catalyst in monolignol polymerization, which builds plant cell walls [23]. According to Silva et al. [24], the activity of the peroxidase enzyme can inhibit the pathogen infection process because of the formation of lignin, which inhibits pathogens from entering.

5. Conclusion
It can be concluded that the independent treatment of variety and composition of organic matter affects the severity of the disease, area under the disease development curve, disease suppression index, salicylic acid activity and peroxidase enzyme activity. The highest disease severity occurred in the independent treatments of V2 (38.19%) and B0 (49.25%). The highest AUDPC value was found in treatment V2B0 (513.3 unit), and the highest DSI was found in V3B2 treatment (52.78%). Increased salicylic acid activity and the highest peroxidase enzyme activity occurred in V3B2 treatment, each at 1.34 and 1.21.

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Acknowledgement
The authors extend their gratitude to the Directorate General of Higher Education. Ministry of Research, Technology and Higher Education of the Republic of Indonesia for providing research grant under Hibah STRANAS in the fiscal year 2019 to support this study.