Increase Resistance to Maydis Leaf Blight and Productivity of Maize in Ultisol Soils with A Combination of Organic Material and Biological Agents Biofresh

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ABSTRACT

**Background:** Low nutrient content in ultisol soils and the result of Maydis leaf rot are a major problem in efforts to increase corn productivity in Southeast Sulawesi Province, Indonesia. The use of organic materials and biological agents is one of the solutions in increasing disease resistance and corn productivity in the environmentally friendly ultisol lands. This study aims to assess the effectiveness of the biological agent Biofresh combined with various types of organic material to increase resistance to Maydis leaf rot and maize productivity in ultisol lands.

**Methods:** Research in April-August 2017 in Southeast Sulawesi, Indonesia. With 21 units of experiments using a random block design with 7 treatments in combination of Biofresh with organic material ‘Bokashi’. consists of: B0 = Inorganic fertilizer; B1 = Organic material of cow dung; B2 = Biofresh Biological Agent; B3 = Biofresh biological agent combined with cow dung organic matter; B4 = Biofresh biological agent combination with bokashi cow dung and straw; B5 = Biofresh combination of biological agents with bokashi cow dung and soybean Sitter; B6 = Biofresh Biological agent combination with all Cow, straw and soybean bokashi.

**Result:** The results showed that the combination of Biofresh with organic material in the form of bokashi at a dose of 5 tons ha⁻¹ was very effective in increasing the durability and productivity of corn plants in ultisol land. The combination of Biofresh biological agents with bokashi containing cow’s feses, straw, and soy litter can reduce Maydis leaf blight leaf blight disease by 47.36% and increase production by 53.54%. Disease suppression index is positively correlated with maize crop productivity in Ultisol land.

**Key words:** Biological agents, Maydis leaf blight, Organic material, Ultisol land.

INTRODUCTION

Corn (Zea mays L.) is one of the important and strategic food commodities in Indonesia since it ranks second after rice and besides corn was also used as animal feed ingredients. Along with population growth and the development of the animal feed industry, so does the demand for corn in Indonesia will mutually increase.

Southeast Sulawesi Province is one of the developed areas in Indonesia that focused on corn plantation. Corn productivity in Southeast Sulawesi is around 2,846 tons ha⁻¹. The productivity indeed is still far below the national productivity of 5,178 tons ha⁻¹ (based on BPS or Indonesian Central Statistics Agency, 2016) and not even nearly from the potential yield of hybrid corn plants that could reach 9-13 thousand tons ha⁻¹. The low productivity of corn in Southeast Sulawesi is caused by most likely less fertile land, less intensive cultivation systems, even pests and plant diseases. One of the important diseases on corn was leaf blight caused by Bipolaris maydis. Which at severe infection and transmission rates. These pathogens can cause yield losses of up to 70% - 100% (Mubeen et al., 2017). To control leaf blight of corn, farmers generally use this kind of synthetic pesticides.

The use of synthetic fertilizers and pesticides in agricultural systems might damage the physical, chemical and biological structure of the land, which results in the degradation of the carrying capacity and quality of agricultural land in Indonesia, resulted that land productivity decreases and land conditions become increasingly infertile (Isnaini, 2006; Rachman et al., 2006), the occurrence of environmental pollution (Al-Zaidi et al., 2011) and the presence of chemical residues in agricultural commodities that are harmful that matters consumer’s health (Szpyrka et al., 2016).

To overcome the problem of soil infertility on ultisol land and corn plant diseases, by being aware from the negative impacts of using synthetic chemicals, an appropriate
technology with ecological insight is much needed. The use of organic materials and biological agents as biological fertilizers in crop cultivation can reduce the use of inorganic fertilizers and synthetic chemical pesticides. One of the biological agents that have been widely studied as biofertilizers and biopesticides in food plants is the Biofresh biological agent. Biofresh is a mixture of rhizobacteria: *Bacillus subtilis* ST21e, *B. cereus* ST21b and *Serratia* sp. SS29a which is formulated in a carrier medium which is capable of secreting cellular enzymes: chitinase, cellulase and proteinase; producing IAA phytohormone; dissolve phosphate and fixating N free of air (Khaeruni et al., 2010). The Biofresh biological agents combined with cows manure organic are able to spur growth and control Rhizoctonia stem rot disease in soybean plants in greenhouses (Satrah, 2014). The Biofresh biological agents combined with soy litter organic material and cow manure are able to increase crop yields and induce soybean crop resistance to postular disease (Wijayanto et al., 2017; Khaeruni et al., 2018) and Soybean Mosaic Virus (Khaeruni et al., 2016). This study aims to determine the effectiveness of Biofresh’s biological agents combined with various types of organic matter to increase the productivity and resistance of corn plants against all forms of diseases in ultisol land.

**MATERIALS AND METHODS**

**Experimental design**

This research was conducted in April-August 2017 at the Experimental Garden of the Indonesian Food Crop Protection Institute, Southeast Sulawesi Province, Indonesia. The experiment was carried out in a replicated manner using randomized block design with 7 treatments in combination of Biofresh with organic material ‘bokashi’. including: B0 = Inorganic fertilizer; B1 = Cow manure organic matter; B2 = Biofresh biological agents; B3 = The combination of Biofresh’s biological agents with cow manure organic matter; B4 = The combination of Biofresh’s biological agents with bokashi cow manure and the straw; B5 = Combination of Biofresh Biological Agents with bokashi Cow Manure and Soybean Litter; B6 = Combination of Biofresh Biological Agents with all Cow, straw and soybean Bokashi. Each treatment was repeated 3 times, which in total there were 21 experimental units.

**Experimental land management**

The land is cleaned and processed using a tractor. Soil treatment was carried out 2 times, then 21 treatment plots were made for plots measuring 3.6 m x 6.3 m, with a height of 30 cm. The distance between treatment plots in groups is 30 cm, where groups are 40 cm. After land management was complete, each experimental plot is evenly sprinkled with dolomite lime at a dose of 2.95 kg / plot or equivalent to 1.3 tons ha⁻¹.

**Application of organic ingredients**

Organic material used in the form of Bokashi which has undergone a fermentation process for 2 weeks. The main ingredients of bokashi are adjusted to the treatment of the tested organic material. Specifically: cow manure, rice straw and soybean litter in a ratio of 1: 1 (v / v) or 1: 1 (v / v / v) when using 2 or 3 types different organic ingredients. The application of organic material in the treatment plot was conducted one day after the application of dolomite lime by evenly sprinkle on the surface of the plot in accordance with the treatment, with a dose of 11 kg / plot that is equivalent to 5 tons ha⁻¹.

**Planting**

Planting was conducted one week after application of organic material. Corn seeds are planted by digging as much as 2 hole⁻¹ seeds at a depth of ± 3 cm with a plant spacing of 30 cm x 75 cm, made it in one treatment plot there are 100 clumps of plants.

**Application of Biofresh organic fertilizer**

Biofresh organic fertilizer application was applied to plots B2, B3, B4, B5 and B6 within twice each. The first application is done shortly after planting and is used as a seed cover in a hole plant with a dose of 10 g hole⁻¹, then the second application is carried out at the age of plants 4 weeks after planting (WAP) by sprinkling around the roots of plants at a dose of 25 g of clump⁻¹.

**Application of inorganic fertilizers**

Inorganic fertilizers used were contained of Urea, SP36 and KCl fertilizer with recommended dosage of Urea fertilizer 200 kg ha⁻¹, SP36 kg ha⁻¹ and KCl 85 kg ha⁻¹ (Khaeruni et al. 2015) B0 trial plot was given 100% of the recommended dose, while other treatments are given a dose of 50% of the recommendation. SP36 Fertilizer is given shortly after planting the seeds by making a hole with a distance of ± 5 cm from the seed hole, Urea fertilizer is given twice, specifically at the age of 2 and 5 WAP, while KCl fertilizer is given at the age of 2 WAP.

**Plant maintenance**

Plant maintenance includes replanting, weeding, planting and watering. Replanting is done a week after planting, by replacing dead plants or those with poor growth. Weeding is done by pulling or cutting weeds that grow around the plantations at the age of 3 and 6 weeks after tiling/after there are weeds that grow. Soiling is carried out at the age of 6 weeks after tilling together with a second wedding. Watering was conducted as needed if it doesn’t rain.

**Observation variable**

**Severity of leaf blight disease**

Observation of the severity of Maydis leaf blight was recorded on 10 plant samples per treatment, at ages 2, 4, 6, 8 and 10 WAP, by observing and identifying disease symptoms that appeared in corn plants due to natural pathogenic infections and calculated using the following formula:

\[
I = \frac{\sum(n_i, v_i)}{NZ} \times 100\%
\]
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Where
I = Attack Intensity (%);
i = Number of sample plants with damage scale vi;
v = Scale damage value for example to -I;
N = Number of plant samples observed;
Z = scale value of highest damage.

The numerical value of the attack category was measured according to the scale of the Directorate of Food Crop Protection, Ministry of Agriculture R.I (2015), where resulted 0 = no attacks; 1 = area of symptoms in leaves d” 5%; 3 = area of symptoms in leaves> 5–25%; 5 = area of symptoms in leaves> 25-50%; 7 = area of symptoms in leaves> 50-75%; 9 = Area of symptoms in leaves> 75-100%.

Area under Disease Curve Progress (AUDCP) is calculated to determine the overall disease progression. According to Paraschivu and Cotuna (2013), AUDCP is calculated using the formula:

\[
\text{AUDCP} = \sum_{i=1}^{n-1} \left[ \frac{X_i + X_{i+1}}{2} \right] (t_{i+1} - t_i)
\]

Where:
yi = first observation data Number-I;
yi + 1 = observation data i-1 + 1;
ti = Time of the first observation;
ti + 1 = I-1 + 1 observation time.

From the results of the AUDCP, the disease suppression index (DSI) was calculated, which is the level of effectiveness in controlling a treatment for the disease progression. According to Nawangsih et al., (2014), DSI is calculated by the formula:

\[
\text{DSI} = \frac{\text{Dic} - \text{Dib}}{\text{Dic}}
\]

Where
Dic = AUDCP on controlling.
Dib = AUDCP on treatment.

Crop Harvest
Observation of crop yield variables was carried out on 10 plant samples per treatment, by weighing and calculating: weight per wet cob (g) and production of ha\(^{-1}\) (tons).

Table 1: The severity of Maydis leaf blight on corn plants in treatment combination of Biofresh biological agents with different organic materials.

| Treatment | Disease severity at Week After Planting (WAP) |
|-----------|-----------------------------------------------|
|           | 4 WAP | 5 WAP | 6 WAP | 7 WAP | 8 WAP | 9 WAP |
| B0        | 4.2c  | 9.3e  | 14.8c | 15.6c | 16.3d | 16.3d |
| B1        | 4.1c  | 7.8d  | 11.1b | 11.1b | 11.8c | 11.8c |
| B2        | 3.7c  | 6.3c  | 11.1b | 11.1b | 11.8c | 11.8c |
| B3        | 2.6b  | 5.6c  | 10.7b | 10.7b | 11.1bc| 11.1bc|
| B4        | 1.8b  | 4.1b  | 10.4b | 10.4b | 10.7b | 10.7b |
| B5        | 1.1ab | 3.7b  | 9.6ab | 9.6ab | 10.2ab| 10.2ab|
| B6        | 0.7a  | 2.2a  | 8.9a  | 8.9a  | 9.3a  | 9.3a  |

Note = B0: Inorganic fertilizer NPK, B1: Cow manure organic matter, B2: Biofresh biological agents, B3: Biofresh’s biological agents + Cow manure organic matter, B4: Biofresh’s biological agents + bokashi Cow manure and straw, B5 = Biological agent biofresh + bokashi Cow Manure and Soybean Litter, B6: Biological agent biofresh + Cow, straw and soybean Bokashi.

RESULTS AND DISCUSSION
Severity of leaf blight disease
The combination of Biofresh’s biological agents with different bokashi organic materials significantly affected the severity of Maydis leaf blight on corn (Table 1). Maydis leaf blight symptoms begin to appear at the age of 4 weeks after planting. The lowest disease severity level at the observation since the 4th to 8th week after planting is found in the combination of Biofresh biological agents with bokashi from cow’s manure, rice straw and soybean litter (B6 treatment). The severity of leaf blight in the B6 treatment was significantly different from the control (B0) and treatments that only used Biofresh biological agents or organic matter (B1 and B2) and combination treatments that did not use soy litter organic materials (B3 and B4). The severity of Maydis leaf blight on B6 treatment at the age of 8 weeks after planting ranged from 9.3%, while other treatments of disease severity was above 10%.

Area under Disease Curve Progress (AUDCP) and Disease Suppression Index (DSI)
The development analysis of Maydis leaf blight during the observation was conducted by calculating the value of AUDPC as shown in Fig 1. All treatments tested both organic material or biological agents individually or a combination of the two, can reduce the AUDPC value, this is indicated by the AUDPC value on the control (B0) higher than other treatments.

The combination of Biofresh biological fertilizer treatment with bokashi containing cow’s manure, straw and soy litter (B6 treatment) has the lowest AUDPC value of...
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240% / day, followed by a combination of Biofresh biological agent treatment with bokashi containing cow manure and soy litter with AUDPC 269% / day, while plants that are only given inorganic fertilizer 100% of the recommended dose (B0 treatment) has an AUDPC value of 463% / day.

The results of the calculation of the disease suppression index showed that the treatment of a combination of biofresh biological agents with bokashi (B6, B5, B4 and B3) is always lot powerful in suppressing disease progression rather than a single bokashi treatment (B1) and a single Biofresh biological agent (B2) as shown in Table 2.

The treatment using Biofresh biological agents combined with bokashi organic feces of cattle, rice straw and soybean litter (B6 treatment) had the highest suppression index for the development against Maydis leaf blight disease which is 47.36%, followed by treatment using a combination of Biofresh biological agents with organic material from cow feces with soy litter (B5 treatment) with a value of 41.03%.

Productivity

The observation results showed that the highest production was obtained by a combination of Biofresh biological agents with bokashi containing cow’s feces, rice straw and soybean litter (B6 treatment) with the weight of the seeds per kernel and productivity respectively 87.4 and 7.77 tons ha⁻¹. Productivity along the B6 treatment was significantly different from other treatments, except for the B5 treatment which was not significantly different (Table 3). When compared with the productivity of 100% inorganic fertilizer treatment or control (B0 treatment) which has a productivity of 3.61 tons ha⁻¹, the B5 treatment and B6 treatment increases productivity by more than 50%.

Regression analysis of diseases suppression index versus products

The results of the regression analysis of the relationship between the suppression index of Maydis leaf blight disease to the production of corn plants treated with Biofresh biological agents with different organic matter are shown in Table 4.
Simple regression equation of disease suppression index (X) against production (Y) = 2.426 + 0.103X, shows that if there is no disease suppression index corn production is only 2.426 tons and every 1% increase in disease suppression index, then production will increase amounting to 0.103 tons / ha, and the coefficient of determination (R^2 = 0.66) describes that 66% increase during production was influenced by the disease suppression index, with a significance of α 0.05, as shown in Fig 2. The data were processed by regression analysis of Microsoft Excel program.

The different composition of organic matter in making bokashi will significantly affect the ability of Biofresh’s biological agent for increasing plant resistance against Maydis leaf blight disease and corn crops productivity in ultisol land. The results of this study indicate the effectiveness of each combination treatment was influenced by the composition and type of organic material that contains the bokashi which was used. All treatments combined Biofresh’s biological agents with organic material (bokashi) capable of increasing plant growth, suppressing the development of Maydis leaf blight and increasing corn crops productivity.

Biofresh Biological Agent is a biological agent based on Southeast Sulawesi's original indigenous rhizobacteria which has the ability as a biofertilizer and biopesticides. Therefore the application of Biofresh’s biological agents combined with more complete organic material with the right composition able to increase the population of these bacteria on the soil following directly and indirectly increase disease resistance and plant productivity. Wahyuni (2016) states that the provision of organic material cow manure can increase the bacterial population from 102 to 107 cfu / gram of soil, compared to controls. Bonanomi et al. (2007) noted a number of studies that show which the application of organic materials significantly reduces the contagiousness of soil infectious diseases because organic materials contain fungitoxic compounds which directly affect the life of pathogens in the soil.

The increase of corn plants resistance is characterized by the lower disease severity of Maydis leaf blight on plants that are applied by a combination of biological agents Biofresh with bokashi containing cow feces. Soybean litter and rice straw compared to the control or sole treatment of biological agents or organic matter. The treatment effectively inhibited disease progression with a disease suppression index value of 47.36%. The results of this study also reinforce the results of previous studies which showed that the application of Biofresh biological agents with soy litter organic material was able to suppress the development of bacterial pustule disease in soybean plants Khaeruni et al. (2018). The increase to capability to suppress the development of disease the application of a treatment combination of Biofresh biological agents with bokashi which contains organic feces of cattle straw and soybean litter also enhances crop productivity by 53.54% compared to controls. Bokashi derived from manure and plant residues contains nutrients and organic matter that can improve the physical features, chemical and biological of the soil. Thus affecting the growth of root and its development as well as the ability of roots to absorb nutrients which greatly determines the vegetative growth of plants which ultimately determines the reproductive phase and yield of plants. Organic matter could form complex compounds with metal ions that are capable of spreading poison plants such as Al, Fe and Mn which is also an energy source for the life of soil organisms that carry out various important processes in the soil (Suriadikarta et al., 2006).

The legumes group belongs to a high quality source of organic matter because it contains a high N > 2.5% the C / N ratio is low (< 20) low lignin content (< 15%) and low polyphenols (< 4%) making it easy to decompose by microorganisms in the oil (Rachman et al., 2006). In line with the research of Tola et al. (2007) showed that the use of bokashi fertilizer with a dose of 20 tons ha^{-1} gave the best results on the growth and production of corn plants. Arinong et al., (2008) revealed that in between treatments using bokashi and without bokashi were significantly different matters. The combination of Biofresh biofertilizers and organic matter can increase plant resistance and crop yields were an accumulation of the ability from three constituent bacteria Biofresh (Bacillus subtilis ST21e. B. cereus ST21b and Serratia sp. SS29a) which besides being able to secrete cellular enzymes such as chitinase, cellulase and

**Table 3**: Production of corn plants that received a treatment combination of Biofresh biological agents with different bokashi materials.

| Treatment | Seed Weight/ Kernel (g) | Productivity (ton.ha^{-1}) | Increasing (%) |
|-----------|-------------------------|-----------------------------|----------------|
| B0        | 40.6a                   | 3.61a                       |                |
| B1        | 41.8a                   | 3.72a                       | 2.95           |
| B2        | 42.6a                   | 3.79a                       | 4.78           |
| B3        | 52.0b                   | 4.62b                       | 21.86          |
| B4        | 79.0c                   | 7.02c                       | 48.57          |
| B5        | 86.1d                   | 7.65d                       | 52.81          |
| B6        | 87.4d                   | 7.77d                       | 53.54          |

**Table 4**: Recapitulation of the results of the regression analysis; DSI Vs Production.

| Model     | Unstand. Coeff. | Summary | AnovaRegresi |
|-----------|-----------------|---------|--------------|
| Constanta 1 |                 |         |              |
| DSI (%)   | .2426           | 0.81    | 0.66         |
| Sig.      | .026            | 1233    | 1937         |
| F hit.    | 9.78            | 4.38    | 0.026        |
| F tab.    | 3.1             | 0.026   |              |

F tabel (α 0.05; db1; db2) = 4.38

**Note**: Sig*. 0.026 < α 0.05* Significant.

Devendentvariable Production Tons^{-1}. DSI = Disease Suppression Index (%).
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proteinase) and play a primary role in various antagonistic mechanisms that also capable to produce IAA phytohormone and dissolve phosphate which generates the driver of plant growth (Khaeruni et al., 2010) and elicitors that impact systemic resilience in plants (Khaeruni et al., 2015; Khaeruni et al., 2016; Khaeruni et al., 2018).

The combining ability of Biofresh’s biological agents with organic material to suppress the development of Maydis leaf blight is greatly influenced by the type and composition of the bokashi material used. The more complex the bokashi, follows higher the index of suppression of plant diseases. The higher the disease suppression index, results in higher productivity of corn plants. Consequently, the disease suppression index is positively correlated with productivity with a value of $R^2 = 0.66$, it means that the increase in corn production from this study was 66% influenced by the ability of Biofresh’s biological agents combined with organic matter in suppressing the severity of Maydis leaf blight caused by the fungus Bipolaris maydis and the remaining 34% was influenced by the direct role of the organic material in increasing production.

CONCLUSION

The Biofresh applied together with organic material in the form of bokashi is very effective in increasing the durability and productivity of corn in ultisol lands. Biofresh biological agents with bokashi containing cow’s feces combined with biological treatment straw and soybean litter is the best treatment that is able to suppress Maydis leaf blight with a disease suppression index of 47.36% and increase production by 53.54%. Disease suppression index is positively correlated with corn crop productivity in Ultisol Lands.

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