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MORPHOLOGICAL CHARACTER AND CLOROPHYL CONTENT INDEX OF CORN INFECTED WITH DOWRY DISEASE ON LAND APPLIED WITH SLOW REALEASE FERTILIZER BASED ON CORN COB BIOCHAR

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Abstract
Downy mildew is a significant disease of corn plants caused by the pathogenic fungus Peronosclerospora maydis, with attack rates reaching 95%. The application of slow-release fertilizer based on corn cob biochar is expected to increase the resistance of corn plants. Similarly, Bacillus sp. and Pseudomonas sp. are known as antagonistic microorganisms. Bacteria can produce antibiotic compounds that hydrolyze fungal cell walls, siderophores, and other antibiotic properties that inhibit pathogen growth. This study aims to determine the morphological characteristics of downy mildew-infected corn on biochar-applied land. Treatments were arranged factorially in a Factorial Randomized Block Design repeated four times. The treatment was the application of slow-release fertilizer from biochar mixed with NPK fertilizer, namely control, slow-release fertilizer, slow-release fertilizer + cow urine, and slow-release fertilizer + cow urine + bacteria (Azobacter and Bacillus). The results showed that slow-release fertilizer gave the best growth to both normal and downy mildew-affected maize plants. The stomata of typical corn leaves were more open with regular vascular bundles, while those affected by downy mildew had more closed and irregular stomata. The chlorophyll content index in downy mildew-affected maize can also improve by applying biochar-based slow-release fertilizer.

Keywords— Azotobacter, cow urine, downy mildew, stomata

Introduction

Maize is the second largest contributor after rice in the food crop sub-sector (Rustiani, 2015). The Maize Commodity Balance Prognosis estimates domestic maize production in 2023 to reach 18.15 million tons, with carryover stocks from 2022 at 3.08 million tons. Meanwhile, maize demand throughout 2023 is estimated at 16.98 million tons. However, according to data from the Directorate General of Livestock and Animal Health of the Ministry of Agriculture, there are 3,951 farmers spread across seven provinces and 40 central districts with a population of 17.5 million heads with an estimated total maize allocation required of 63,327 tons (Statistics Indonesia, 2018). It indicates a higher demand for maize. Efforts can be made to increase maize

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production in Indonesia using superior seed varieties, such as hybrid maize. Hybrid maize is very responsive to fertilization, so its production potential is also high at 10-12 tons/ha (Hastini & Noviana, 2020).

There are several inhibiting factors in efforts to increase corn production, one of which is the attack of plant disruptors that cause downy mildew by the fungus P. maydis. Typical symptoms of downy mildew are chlorosis spreading parallel to the leaf veins, stunted plant growth, and, in the morning, a white powdery coating under the leaf surface (Jatnika et al., 2013). Corn plants infected with P. maydis can experience a reduction in production of 80-100%. Corn plants infected with P. maydis cannot produce seeds (Ridwan et al., 2015). Infected corn plants are still very young and usually have not yet borne fruit, causing crop failure (Ulhaq & Masnilah, 2019). There are three downy mildew species: Peronosclerospora maydis, P. philippinensis, P. sorghi. P. maydis attacks maize plants in Kalimantan and Java, P. sorghi in Sumatra, and P. philippinensis in Minahasa, North Sulawesi (Hikmawati et al., 2011). Therefore, efforts to increase corn production must be done in various ways, such as preparing a good place to grow and fertilizing or providing maximum nutrients. It can be utilized on dry land for sustainable agricultural development. The cultivation system can increase soil productivity and reduce the rate of soil degradation. It can improve soil quality in situ, especially by improving the soil's physical, chemical, and biological properties (Utomo, 2015).

One solution for fertilizing maize crops in drylands is the application of slow-release fertilizers that have negligible nutrient solubility and can provide nutrients gradually (continuously) over a long period. Slow-release fertilizer improves nutrient absorption, and nutrient loss due to leaching will be less severe (Sagala 2018). This slow-release fertilizer can be obtained by mixing chemical NPK fertilizer with biochar.

Biochar can be used as a soil improver and can raise soil pH (Solaiman and Anawar, 2015); this is related to the role of biochar, which can increase water and nutrient retention. The physical characteristics of biochar, such as surface area, shape, structure, and porosity, play an essential role in soil water retention (Andrenelli et al., 2016; Liu et al., 2017), nutrient retention, and aeration (Drahansky et al., 2016). In addition, biochar can improve soil chemical properties such as pH (Agegnehu et al., 2017).

Research Method

The research was conducted on farmers' land in Parenting Village, Lilirilau District, and Soppeng Regency. Lat -4.343827º, long 120.050652º.

The materials used were hybrid corn seeds of Bisi-2 variety, corn cob biochar, slow-release fertilizer (combination of compound NPK + corn cob biochar), urea fertilizer, cow urine, manure, Bacillus, and Azatobacter bacteria. At the same time, the tools used in this research were cultivar machines, hoes, shovels, meters, digital scales, rulers, vectors, labels, raffia, tugal tools, stationery, and ropes.

The research was organized using a completely randomized design (CRD) consisting of 4 treatments and three replications, namely:

P0= No treatment
P1= slow release
P2= slow release + Cow Urine
P3= slow release + Cow Urine + Bacteria

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Figure 1. Research location Parenring Village, Soppeng Regency, South Sulawesi

Biochar production

Corn cob biomass was obtained from the farmer's field after harvesting. The dried corn cobs were put into a closed drum. A fire was lit under the drum for approximately three hours. When the corn cobs are entirely black, the burning process has been completed. The charcoal is removed from the drum and then doused with water to prevent it from becoming dust. The cooled corn cobs are chopped and then put into sacks.

Manufacture of slow-release fertilizer

Burning is done using easy tools and procedures for farmers to use. Biomass is dried by drying in the sun. This is done so as not to produce smoke when burned. Combustion is carried out under anaerobic conditions using a drum for 4 hours. The combustion temperature is about 100°C. The appearance of a blue flame indicates that the combustion process has been completed. After burning, the corn cobs are then put into sacks and continued with shredding. Burning 25 kg of corn cob can produced biomass produces 5 kg of corn cob biochar. Slow-release fertilizer begins with mixing corn cob biochar with phonska fertilizer, then stir until well mixed. After mixing, then put it in a plastic sack. Leave it for about a week, then apply it to the field.

Observation Parameters

The observation parameters in this study were measured 8 days after planting, namely plant height (cm), stem diameter (mm), number of leaves (strands), chlorophyll content index, chlorophyll content index a, chlorophyll content index b. The chlorophyll content index follows Equation 1 according to (Goncalves, 2008), namely:

$$y = a+b\,(cci)c \quad \text{..... (1)}$$

y is the chlorophyll value, a is the constant value of a, b is the constant value of b, and c is the constant value of c. The value of chi a (a)=-421.35 (b)=38.23, the value of chi b (a)=375.02 (b)=4.03, and the value of chi c (a)=0.1863 (b)=0. The CCI value was measured using the chlorophyll concentration index tool by taking leaves attached to branches that produce fruit. One leaf was taken from each treatment, so there were 45 leaves for CCI measurement.

In addition, the N-total, K, and C-organic content were analyzed using the Kjehdahl

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method, which consists of deconstruction, distillation, and titration. Observation data were analyzed using the SAS (Statistical Analysis System) program and DMRT (Duncan's Multiple Range Test) further tests.

Results and Discussion

Morphological and Stomatal Characteristics of Maize Leaves Affected by Downy Mottle Disease

Maize infested with downy mildew by the pathogen P. maydis is characterized by a white powdery coating under the leaf surface and chlorosis (Figure 2).



Figure 2. Normal maize plants treated with NPK as control (A), plants affected by downy mildew (B), and white powder on maize leaves showing symptoms of downy mildew (C).

Chlorosis in downy mildew is characterized by pale stripes parallel to the leaf bones. Microscopically, the fungus P. maydis has a conidiophore shaped like a rod; at the end of the rod, there are round conidia. According to Ridwan et al. (2015), the initial symptom of downy mildew is the appearance of yellowish lines (chlorosis) parallel to the leaf bone. Then, chlorosis spreads over the entire leaf surface. There are conidiophores shaped like rods, and then there are spores or round conidia on the branches at the ends (Jatnika et al., 2013). Healthy plants have no chlorosis symptoms on the leaves, characterized by pale lines parallel to the leaf bones. The color difference is evident between healthy leaves and leaves attacked by downy mildew during the incubation period, which is the time between the onset of infection and the onset of symptoms. Plants resistant to the disease will show a more extended incubation period than susceptible plants.

Jatnika et al. (2013) reported the results of observations of the incubation period of the pathogen P. maydis on Pioneer 21 varieties showed symptoms that appeared when the plants were 7 days after inoculation. Research by Ridwan et al. (2015) showed that symptoms of P. maydis on Bonanza varieties appeared on day 5 after inoculation. Turnip et al. (2015) showed that P. maydis symptoms appeared 2 days after inoculation. The varying incubation periods occur due to several influencing factors, such as pathogen virulence, host resistance, and environmental conditions, such as temperature and humidity, that support pathogen development.

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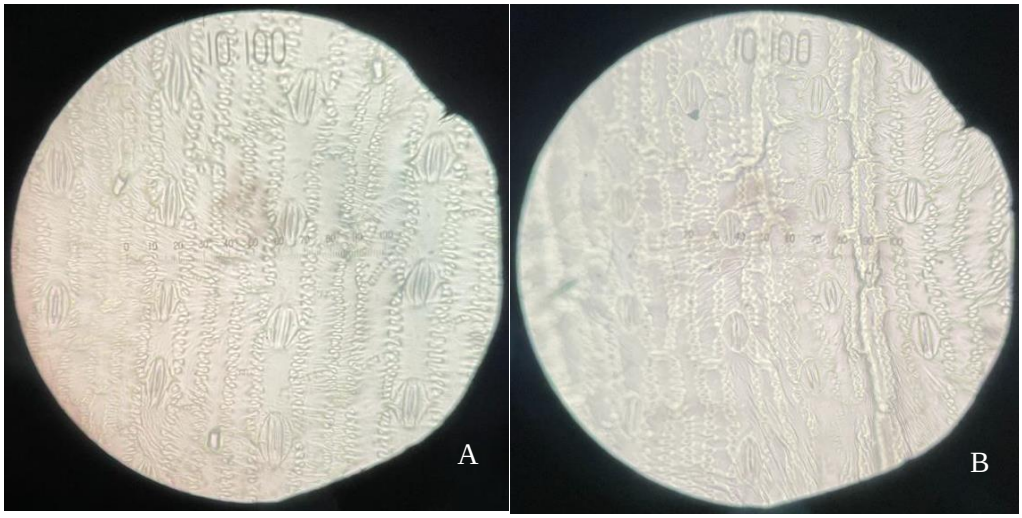


Figure 3. Stomata of regular corn leaves (A) and plants affected by downy mildew (B).

The addition of P. fluorescens to the treatments caused lower disease severity. It is closely related to the antagonistic mechanism of P. fluorescens in the mechanism of nutrient competition, antibiosis, and induced resistance (Nasrun & Burhanuddin, 2016). Jatnika et al., (2013) also reported that the application of P. fluorescens can reduce downy mildew attack by 33 to 50%. There are also differences in stomata on the leaves of typical and downy mildew-affected corn plants. The stomata of normal maize have a more regular arrangement of stomata and vascular bundles (Figure 3A). In contrast, the arrangement of stomata of downy mildew-affected maize looks irregular, tending to be covered with untidy bundles (Figure 3B). It causes a lack of nitrogen supply to the plant through the leaves. Pramitasari et al. (2016) stated that nitrogen supply dramatically affects the growth of plant vegetative organs. The number, length, width, and density of corn leaf stomata were highest in the slow-release fertilizer + cow urine + bacteria, while the stomatal opening area was highest in the control treatment (Table 1).

Table 1. Average size, density, and stomatal opening area (mm) of normal and downy mildewed maize plants after slow release fertilizer application at 8 weeks after transplanting

Fertilizer combination ***Slow- release***	Corn Leaf Stomata				
	Total	Lenght	Width	Density	Area of Opening
Control (p0)	13	23	4	0,64	72,22
Slow-release fertilizer (p1)	14	17	2	1,37	26,06
Slow-release fertilizer + Cow urine (p2)	11	26	3	0,91	61,23
Slow-release fertilizer + Cow urine + bacteria (p3)	16,5	23	2,5	1,30	41,21

Growth of downy mildew-affected maize applied with Slow Release Fertilizer

The growth of normal maize plants (not affected by downy mildew) compared to plants affected by downy mildew is shown in Table 2.

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Table 2. Average stem diameter, number of leaves, and height of typical and downy mildewed maize plants observed for 8 weeks after transplanting after slow-release fertilizer application.

Fertilizer combination ***Slow- release***	Stem Diameter (cm)		Number of leaves (leaf blade)		Plant height (cm)	
	Normal	downy mildewed	Normal	downy mildewed	Normal	downy mildewed
Control (p0)	2,04 [a]	0,59 [a]	7.4 [b]	4.34 [a]	131.60 [b]	39.43 [b]
Slow-release fertilizer (p1)	2,50 [a]	1,61 [a]	11.13 [a]	4.40 [a]	185.00 [a]	66.96 [a]
Slow-release fertilizer + Cow urine (p2)	2,10 [a]	1,13 [b]	12.10 [a]	4.10 [a]	203.53 [a]	69.07 [a]
Slow-release fertilizer + Cow urine + bacteria (p3)	2,10 [a]	0,74 [c]	11.40 [a]	3.62 [a]	162.73 [ab]	34.88 [b]

Notes: Numbers followed by the same letter are not significantly different in the DMRT α 5% test.

Table 2 shows that in typical plants (TN) and plants affected by downy mildew, slow-release fertilizer has better plant growth than the control. Slow-release fertilizer is a combination of soil improver (biochar) that can maximize soil physical properties for growth and compound NPK, which plays an important role in the availability of nutrients in the soil to support plant growth and production. The best growth is achieved by applying slow-release fertilizer added to cow urine. However, its effect on plant growth takes longer than chemical fertilizers. It is due to the slow nature of biological organic fertilizers, especially at the beginning of growth (Gubali, et al., 2015).

The results in this study align with the research of Usodri et al. (2021), which showed that the application of slowrealese fertilizer optimized the growth of hybrid corn seedlings in the main nursery on all observed observation variables. In addition, Hutapea et al. (2014) added that slow-release fertilizer could spur the growth of downy mildew-infected corn on the growth variables of stem diameter, number of leaves, and plant height. The role of compound slow-release fertilizer as another combination fertilizer also has a high enough nutrient content that can help prevent and increase disease resistance. Wijayanto and Sucahyono (2019) stated that plants that lack K elements are more easily infected. Plant height and number of leaves are closely related to wet weight. If the plant height and number of leaves increase, the wet weight of the plant will increase.

Organic matter in the form of corn cob biochar is one of the ameliorants that improves soil fertility despite its slow decomposition activity. This slow decomposition activity causes this organic material to have slow-release properties. Compost, classified as a slow-release fertilizer, will release the nutrients contained slowly and continuously over a certain period so that water leaches nutrients less. Organic matter provides nutrients for plants, has macro and micro pores so that air circulation is quite good and high water absorption, and improves the physical properties of clay soil (Hayati et al., 2012; Sondakh et al., 2017; Damanik et al., 2017). Improvement of soil physical properties can increase the quality of soil porosity, and the ability of soil to retain water increases.

Chlorophyll Content Index of downy mildew affected corn plants

The chlorophyll content index of downy mildew-affected plants applied with slow-release fertilizer is presented in Table 3.

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Table 3. Average Chlorophyll Content Index (CCI) a and b of corn leaves in normal and downy mildew-affected plants after slow-release fertilizer application observed 8 weeks after transplanting.

Fertilizer combination ***Slow- release***	*Chlorofil Content Index*		*CCI a*		*CCI b*	
	Normal	downy mildewed	Normal	downy mildewed	Normal	downy mildewed
Control (p0)	20,22 ^{a}	13,11 ^{ab}	344,3 ^{a}	622,2 ^{ab}	54,97 ^{a}	330,1 ^{ab}
Slow-release fertilizer (p1)	25,49 ^{a}	12,93 ^{a}	955,4 ^{a}	563,7 ^{ab}	64,31 ^{a}	315,9 ^{ab}
Slow-release fertilizer + Cow urine (p2)	21,62 ^{a}	10,36 ^{b}	777,7 ^{a}	361,0 ^{b}	57,45 ^{a}	213,1 ^{b}
Slow-release fertilizer + Cow urine + bacteria (p3)	21,04 ^{a}	15,87 ^{a}	524,3 ^{a}	878,7 ^{a}	56,42 ^{a}	475,9 ^{a}

Notes: Numbers followed by the same letter are not significantly different in the DMRT α 5% test.

The slow-release fertilizer for CCI, CCI a, and CCI b parameters was higher than the control (Table 4). Chlorophyll is an essential indicator for health and evaluation of plant photosynthetic ability and growth status. Leaf chlorophyll levels are related to plant condition, so they can be used to determine the additional fertilizer plants need. Chlorophyll measurement is one way that can be used to measure the level of growth and fertility of plants, which can be associated with predictions of production from plants (Putri et al., 2016).

According to Hidayah et al. (2019), chlorophyll levels in plants decrease and increase in line with nutrient adequacy. The level of plant development and N translocation influences the dynamics of leaf CCI. (2019) showed that chlorophyll levels increased with increasing plant age until the leaves were fully developed, then decreased as the leaves aged. Furthermore, Setiawati et al. (2016) stated that the CCI of plant leaves is linearly correlated with leaf chlorophyll levels. While plants are affected by downy mildew, according to Jabeen et al. (2017), there is a more significant reduction in chlorophyll a and b content in susceptible plants, followed by moderate plants and then resistant plants. According to Mishra et al. (2015), reducing chlorophyll content due to plant disease affects photosynthesis. The presence of pathogens can interfere with plant physiology, one of which is a decrease in the amount of chlorophyll, even though the environment is in an optimum state for plants (Agustamia et al., 2016). As shown in Table 4, typical plants' CCI is higher than downy mildew-affected plants. Chlorophyll content itself is one indicator of the photosynthesis process in plants.

N, P, K, and C-organic content of Maize Plant Tissue

The results of NPK and C-organic analysis on hybrid corn plant tissues showed that the application of slow-release fertilizer was not significantly different between treatments (Table 4).

Table 4. Average NPK and C-organic content of corn plant tissues in normal and downy mildew-affected plants after slow-release fertilizer application.

Fertilizer combination ***Slow- release***	N Total (%)	P (%)	K (%)	C-Organics
Control (p0)	0,49	1,49	1,95	31,41
Slow-release fertilizer (p1)	0,48	0,24	1,99	33,97
Slow-release fertilizer + Cow urine (p2)	0,50	0,19	1,92	33,38
Slow-release fertilizer + Cow urine + bacteria (p3)	0,66	0,19	1,18	33,77

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However, total N levels were lower in the slow-release fertilizer without adding cow urine and bacteria. It was in contrast to P and K levels, which were higher in only slow-release fertilizer without adding urine and bacteria. It is in line with the statement of Gandahi et al. (2015) that biochar encourages mechanisms that increase plant growth and nutrients. The use of biochar can increase the N content in the soil. One of the benefits of the N element for plants is to stimulate the vegetative growth of plants, such as stems, leaves, and roots. According to Beah et al. (2015), compost fertilizer can increase N uptake, which will increase as the application of organic fertilizer increases. The relationship of C-organic with total plant N and plant N uptake, according to Wijanarko et al. (2012), is an essential process for the availability of N in the soil. According to Kastalani et al. (2017), N is associated with the formation of chlorophyll in the leaves, so it will increase the photosynthesis process and spur the growth of the number of leaves in plants. In line with Supramudho et al. (2012), the higher the total N content of the soil, the higher the N uptake by plants. The higher the application of organic matter into the soil, the more the N uptake by plants will increase.

Conclusion

The use of slow-release fertilizer on corn plants can increase the growth of both normal or healthy plants and diseased plants. The addition of cow urine and bacillus bacteria increases the resistance and growth of downy mildew-affected corn. Chlorophyll Content Index (CCI) in normal corn plants (not affected by downy mildew) is highest in applying slow-release fertilizer, namely 25.49. In corn affected by downy mildew, the highest is in slow-release fertilizer + cow urine + bacteria, namely 15.87. Plants affected by downy mildew.

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