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Effect of Bacterial Consortium and Rice Husk Charcoal to Control Downy Mildew in Corn (*Zea mays* L.)

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ABSTRACT

Background: The purpose of applying bacterial consortium and rice husk charcoal to corn plants is to determine the effect of the combination and whether it can control the intensity of downy mildew attacks and increase corn plant growth.

Methods: This research was conducted at the experimental farm of the University of Muhammadiyah Malang. This research was conducted using two factors: the dose of rice husk charcoal application and the application of bacterial consortium with three replications using a randomized block design. The treatments were Charcoal husk (H): H5 = Charcoal husk @5 tons/ha, H10= Charcoal husk @10 tons, H15 Charcoal husk @15 tons/ha, Bacterial consortium (W): W0= without bacteria, WL = bacterial consortium 10 cc/l *via* foliar spraying, WS = bacterial consortium 10cc/l through soil, WLS = bacterial consortium 10cc/l through soil and foliar spraying

Result: The results showed that the intensity of downy mildew attack on the combined application of the bacterial consortium and rice husk charcoal was lower than that of the no-combination treatment. The length and diameter of the plants that were treated with rice husk charcoal had a significant effect. Still, the application of the bacterial consortium had no effectand the number of leaves was not affected by the treatment.

Key words: Bacteria, Bacterial Consortium, Corn, Downy mildew.

INTRODUCTION

One of the obstacles in cultivating corn is downy mildew caused by the fungus *Peronosclerospora*. Downy mildew is one of the primary diseases of maize caused by *Peronosclerospora philliphinensis.* Plants infected with downy mildew will not give optimal results. Typical symptoms of downy mildew disease in corn plants are chlorosis extending parallel to the veins of the leaves, stunted growth of the affected plantsand a layer of white powder under the surface of the leaves in the morning. Downy mildew pathogens encompass various species of obligate oomycetes, posing severe threats to cultivated, ornamental and indigenous plant populations (Salcedo *et al.,* 2021). Species such as *Peronosclerospora sorghi*, P. *philippinensis*, *P. sacchari*, *P. spontanea*, *Sclerophthora rayssiae var. zeae* and *P. maydis* have caused substantial losses due to widespread downy mildew outbreaks worldwide (Janruang and Unartngam, 2018). Corn plants infested with *P. maydis* can experience a reduction in production of 80% -100%. Corn plants attacked by *P. maydis* cannot produce seeds (Herlina, *et al.,* 2017).

Control of downy mildew disease generally uses seed treatment with synthetic fungicides. However, the excessive use of synthetic fungicides can result in the emergence of novel strains and outbreaks within agricultural environments, thereby adding complexity to disease control efforts. Consequently, relying on synthetic fungicides over prolonged periods is not environmentally sustainable and may prove ineffective as a long-term strategy for mitigating downy mildew disease (Wu *et al.,* 2023). The continued use of synthetic fungicides is known to have a negative impact on non-target organisms and the soil. Using biological agents is a solution to eliminate fungicides in combination with resistant varieties (Najamuddin *et al.,* 2023). Other control techniques with resistant varieties continue to be developed. However, no varieties have been reported to be highly resistant to downy mildew attack (Arulselvi and Selvi, 2021). There is a need to develop other control methods, such as bacteria.

Using rhizobacteria is an alternative to control downy mildew in corn plants caused by *Peronosclerospora maydis*. Rhizobacteria are a group of bacteria with a habitat in the plant root area (rhizosphere) which has been widely researched and proven to increase soil fertility, increase plant resistance and suppress plant pathogens (Wang *et al.,* 2021). Rhizobacteria act directly as biofertilizers and bio-stimulants

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by producing plant growth hormones such as IAA (Indole - 3- acetic acid), gibberellin, cytokinin, ethylene, dissolving mineralsand indirectly also function to prevent pathogenic microorganisms through the formation of siderophores, antimicrobial components (antibiotics) and potentially as an agent in induced systemic resistance (Purnawati, *et al.,* 2023). Applying biological agents to control downy mildew is an environmentally friendly method recently widely promoted. *Trichoderma* sp. and plant growth promoting rhizobacteria (PGPR). The results showed that the combination of *Trichoderma* sp. and rhizobacteria of 60 mL L-1 was able to inhibit the incidence of the disease up to 66.53% and the disease severity up to 89.84% (Purwant, Wahyudiand Hamyana, 2023).

Utilization of bacterial isolates obtained from the Rhizosphere of dry land roots of annual plants (Coconut Rhizosphere (*Cocos nucifera*), Pine Rhizosphere (*Casuarina equisetifolia*), Cassava Rhizosphere (*Manihot esculenta*), Shrub Rhizosphere (*Chamaedaphane*), Sono Rhizosphere (*Dalbergia latifolia*), Banana Rhizosphere (*Musa paradisiaca*) (Sukorini *et al.,* 2023). The results of initial isolation and selection to determine the types of soil bacteria that play a role and have the potential to be developed and explicitly utilized obtained the ten best bacterial isolates from the Rhizosphere of annual plants. The ten bacterial isolates obtained were subjected to a hypersensitive test using tobacco plantsand seven antagonistic bacteria were obtained. This bacterial isolate was then formulated by mixing the seven bacteria with antagonists to form a bacterial consortium. This is because there is a mechanism between bacteria that support each other to inhibit pathogens that attack plants. This is based on the statement (Jahuddin *et al.,* 2022) that bacterial consortia have better inhibitory power than pure isolates of individual antagonist bacteria in inhibiting the development of pathogens. The application of a bacterial consortium will colonize plants in different ways depending on how it is applied; according to (Limbo-dizon *et al.,* 2023), the application of a bacterial consortium must be as close as possible to the plant because the inoculated bacteria can only move a distance of no more than 2 cm from the inoculation location. Incorporating silica derived from rice husk charcoal has been discovered to have a notable impact in preventing harmful fungal infections, specifically downy mildew, which affect corn plants (Senapati *et al.,* 2022). The treatment of giving organic silica derived from rice husk ash was able to suppress downy mildew attacks on corn plants caused by *P. maydis*, with levels only reaching a value of 4.63 and a disease incidence of 11.11%.

MATERIALS AND METHODS

This research was conducted during the rainy season from February to June 2023. It is located in the Agrotechnology Farm of the University of Muhammadiyah Malang at an altitude of 560 meters above sea level, with the coordinates 7°55’04" S 112°35’41" E.

Experimental Setup

This study used a randomized block design (RBD) arranged factorially. There are two factors: The first factor was Charcoal husk. The second factor was bacterial consortium application. The treatment details were: Charcoal husk (H): H5 = Charcoal husk 5 tons/ha, H10= Charcoal husk 10 tons, H15 Charcoal husk 15 tons/ha. Bacterial consortium (W): W0 = Without bacteria, WL = Bacterial consortium 10cc/l *via* foliar spraying, WS =Bacterial consortium 10 cc/l through the soil, WLS = Bacterial consortium 10 cc/l through the soil and foliar spraying

Bacterial consortium preparation

Each bacterium was cultured in a Potato Dextrose Agar (PDA) medium for five days. Then transfer to Potato Dextrose Broth (PDB) medium. The bacteria population was counted using optical density (OD) measuring. Each bacterial isolate was taken in as much as 1 mL and distilled water was put into a cuvette and used as a blank. Suspension The absorbance value was then calculated with a spectrophotometer wavelength of 649 nm (Hyder *et al.,* 2024). Observations were made at four-hour intervals from the start of the observation and carried out for 24 hours.

Land preparation and cultivation practice

The land was cleared of weeds and crop residues with a hoe to loosen the soil. Then, the loose soil was made into elongated beds in an east-west direction. Beds were made with a height of 20 cm, 2.2 m long and 1 m wide and irrigation channels were made 30 cm wide. Sweet corn seeds of the Bonanza F1 variety were planted in the planting holes made using a drill in each plot with a spacing of 70 cm × 40 cm and a depth of approximately 5 cm in each plant hole with two seeds of corn. Fertilization with charcoal husk was carried out at the beginning of planting corn seeds at a dose of five tons/ha (140 g/plant). Ten tons/ha (240 g/plant). Fifteen tons/ha (420 g/plant). Charcoal husk was applied by sprinkling rice husk charcoal in the planting hole.

Provision of the bacterial consortium

Bacterial consortium application was carried out at plant ages 10, 17, 24 and 31 days after application (DAA) with a dose of 10 ml/plant and a bacterial density concentration of 108 cfu/ml (Hadi *et al.,*2021). The bacteria was sprayed on all parts of the corn plants that were still healthy.

Observational variables

Observational variables consist of number of leaves, plant length, stem diameter, cob length, cob diameterand disease intensity (DI). DI was done by observing the infection or damage that occurs in plants. Calculation of the disease intensity using the following formula:

$$I = \frac{a}{b} \times 100\%$$

Where:

I= Intensity of severity of the disease.

a= Number of plants affected by downy mildew.
b= The total number of plants.

Statistical analysis

Statistical analyses of data were carried out for Randomized Block Design. Data were subjected to the analysis of variance and least significant difference at a probability level d" 0.05. The non-significant differences were denoted as ns.

RESULTS AND DISCUSSION

Number of Leaves

There was no interaction effect between the husk charcoal dose and the bacterial consortium application. The application of husk charcoal and the bacterial consortium had no significant impact on the number of leaves observed 7, 14, 21, 28 and 35 days after application (DAA, presented in Table 1).

Plant height

There was no interaction effect between the husk charcoal dose and the bacterial consortium application. Providing husk charcoal had no significant effect on plant height. Likewise, the application of bacterial consortium except at the age of 7 days after application (DAA). They are presented in Table 2.

Stem diameter

There was no interaction effect between the husk charcoal dose and the bacterial consortium application. As presented in Table 3, the provision of husk charcoal and the bacterial consortium had no significant impact on the stem diameter of corn plants.

Cob diameter, cob length and disease incidence

Charcoal husk treatment without bacterial consortium did not affect cob diameter and length. This is in line with the percentage of disease incidence (%). disease incidence

Table 1: Number of leaves of corn plant because of bacteria consortium and rice husk charcoal application on 7, 14, 21, 28 and 35 DAA.

Treatment	Number of leaves of corn plant because of bacteria consortiumand rice husk charcoal application on 7.14.21.28. and 35 DAA				
	7	14	21	28	35
H5	9.00	10.25	14.46	19.71	24.29
H10	8.96	10.79	14.04	19.08	22.96
H15	9.00	11.62	15.54	20.30	24.09
W0	8.94	9.67	14.00	19.33	25.33
WL	9.00	11.22	14.61	18.56	23.72
WS	9.00	11.33	15.00	20.22	22.45
WLS	9.00	11.33	15.11	20.67	23.61

Note: Numbers accompanied by the same letter in the same column are not significantly different based on the 5% Tukey's test. H5 = Charcoal rice husk 5 tons/ha. H10= Charcoal rice husk 10 tons. H15 Charcoal rice husk 15 tons/ha. W0 = Without bacteria. WL = Bacterial consortium 10 cc/l *via* foliar spraying. WS = Bacterial consortium 10cc/l through the soil. WLS = Bacterial consortium 10cc/l through the soil and foliar spraying

Table 2: Plant height of corn plant because of bacteria consortium and rice husk charcoal application on 7, 14, 21, 28 and 35 DAA

Treatment	Plant height (cm) of corn plant because of bacteria consortiumand rice husk charcoal application on 7.14.21.28. and 35 DAA				
	7	14	21	28	35
H5	24.93a	55.29a	68.50a	73.50a	79.01a
H10	29.53a	57.83a	65.73a	79.00a	74.90a
H15	29.51a	60.09a	65.25a	83.50a	87.72a
W0	17.30a	52.33a	71.63a	77.33a	80.5a
WL	28.40b	61.33a	70.33a	77.33a	74.76a
WS	33.89c	59.34a	64.00a	80.00a	81.22a
WLS	32.36c	57.95a	60.00a	80.00a	85.70a

Note: Numbers accompanied by the same letter in the same column are not significantly different based on the 5% Tukey's test. H5 = Charcoal rice husk 5 tons/ha. H10= Charcoal rice husk 10 tons. H15 Charcoal rice husk 15 tons/ha. W0 = Without bacteria. WL = Bacterial consortium 10cc/l *via* foliar spraying. WS = Bacterial consortium 10cc/l through the soil. WLS = Bacterial consortium 10cc/l through the soil and foliar spraying.

Table 3: Stem diameter (mm) of corn plants due to the influence of treatment corn plant because of bacteria consortium and rice husk charcoal application on 7, 14, 21, 28 and 35 DAA.

Treatment	Stem diameter (mm) of corn plants due to the influence of treatment corn plant because of bacteria consortium and rice husk charcoal application on 7.14.21.28. and 35 DAA				
	7	14	21	28	35
H5	9.98a	13.68a	22.51a	31.92a	42.99a
H10	9.59a	12.98a	23.64a	35.89a	40.17a
H15	9.45a	14.51a	23.28a	38.64a	45.36a
W0	11.00a	12.67a	17.23a	25.00a	49.67a
WL	9.24a	12.55a	21.56a	31.99b	49.52a
WS	9.25a	13.41a	25.58a	42.12c	55.82a
WLS	9.20a	16.26a	28.20a	42.83c	59.19a

Note: Numbers accompanied by the same letter in the same column are not significantly different based on the 5% Tukey's test. H5 = Charcoal rice husk 5 tons/ha. H10= Charcoal rice husk 10 tons. H15 Charcoal rice husk 15 tons/ha. W0 = Without bacteria. WL = Bacterial consortium 10 cc/l *via* foliar spraying. WS = Bacterial consortium 10 cc/l through the soil. WLS = Bacterial consortium 10 cc/l through the soil and foliar spraying.

Table 4: Cob diameter and cob length because of bacteria consortium and rice husk charcoal application.

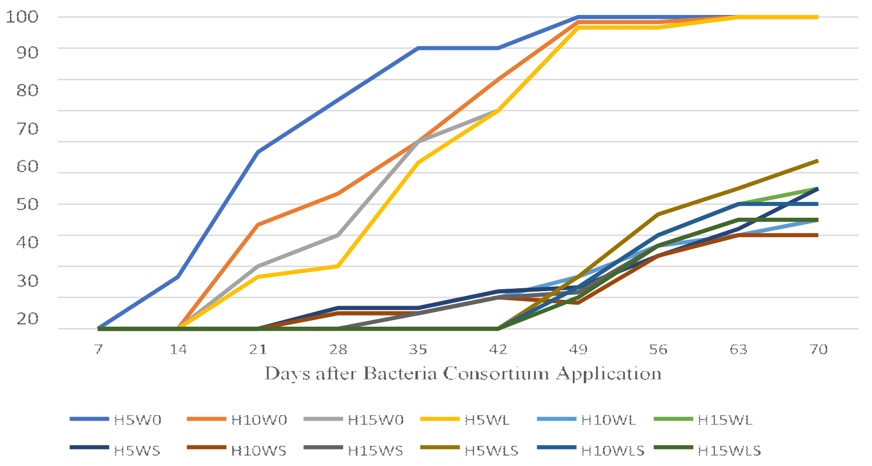
Treatment	Cob diameter (mm)	Cob length (cm)
H5W0	0.00a	0.00a
H10W0	0.00a	0.00a
H15W0	0.00a	0.00a
H5WL	0.00a	0.00a
H10WL	39.92bc	19.39b
H15WL	44.04d	20.39b
H5WS	34.34b	17.82b
H10WS	47.52d	20.56b
H15WS	42.01c	20.28b
H5WLS	46.71d	20.39b
H10WLS	38.49bc	17.97b
H15WLS	43.88c	20.92b

Note: Numbers accompanied by the same letter in the same column are not significantly different based on the 5% Tukey's test. H5 = Charcoal rice husk 5 tons/ha. H10= Charcoal rice husk 10 tons. H15 Charcoal rice husk 15 tons/ha. W0 = Without bacteria. WL = Bacterial consortium 10 cc/l *via* foliar spraying. WS = Bacterial consortium 10 cc/l through the soil. WLS = Bacterial consortium 10 cc/l through the soil and foliar spraying.

occurred in the rice husk charcoal treatment alone without the bacterial consortium. The intensity of the attack reaches more than 90% at the age of the plant 70 days after application. The lowest intensity of attack was in the combination treatment of 15 tons of husk charcoal per hectare and bacterial consortium by foliar and soil spraying. In treatments that were attacked earlier, namely, in treatments without the addition of bacterial consortia, no cobs were produced. This is presented in Table 4 and Fig 1.

The performance of corn plants given rice husk charcoal and bacterial consortium showed a lower attack level than those given rice husk charcoal alone. This has an impact on crop yields. Giving husk charcoal can bind nutrients well to encourage plant growth. Adding husk charcoal to soil media can improve the porosity of soil media, so it is suitable for root respiration and soil moisture (Asfar *et al.,* 2021). According to (Syamsafitri *et al.,* 2023) nutrient content on rice husk charcoal includes several components namely SiO2 (52 Percent), C (31 Percent), K (0.3 Percent), N (0.18 Percent), F (0.08 Percent) and calcium (0.14 Percent, besides that there are also other components such as Fe2O3, K2O, MgO, CaO, Mn and Cu in small quantities. Besides that, rice husk charcoal can maintain environmental conditions and improve the physical properties of the soil, making the water-holding capacity high so that vegetative growth is a better corn crop. The application of rice husk charcoal to the soil, besides being able to be light and high porosity, the soil remains loose, retains soil moistureand stores components nutrients, driving the growth of organisms that are useful for plants and as component anchors nutrient crops when plants are deficient nutrientand husk charcoal is referred to as fertilizer slow release (Bayu Murti Petrus *et al.,* 2020). The ability of the silica component contained in the husk charcoal. The presence of Sufficient silica in the soil can help plants to be more resistant to component imbalances nutrients, which include excess N, deficiency and abundance of Pand poisoning of Na, Fe, Mn and Al (Katz *et al.,* 2021).

The resistance level of maize plants treated by the bacterial consortium also indicated that the intensity of the attack by the pathogen *Peronosclerospora* was low. This is because the bacterial consortium, when formulated, has a disease control mechanism; the treatment of this antagonistic bacterial consortium provides a defense system (bioprotectant) because these bacteria can secrete antibiosis compounds which can encourage attacked plants to fight pathogens (Mugiastuti *et al.,* 2023). This bacterium is thought to be able to produce compounds, such as antibiotic compounds, siderophoresand other secondary metabolites whose properties can slow down fungal activity. Inhibition of pathogens, namely bacteria, can fight against



H5 = Charcoal rice husk 5 tons/ha. H10= Charcoal rice husk 10 tons. H15 Charcoal rice husk 15 tons/ ha. W0= Without bacteria. WL = Bacterial consortium 10 cc/l *via* foliar spraying. WS = Bacterial consortium 10 cc/l through the soil. WLS = Bacterial consortium 10 cc/l through the soil and foliar spraying

Fig 1: Disease incidence (%) of downy mildew disease due to the influence of treatment bacteria consortium and rice husk charcoal application.

the invasion of phytopathogens by producing siderophores. Siderophore is an iron chelating compound or Fe3+ complex made by microorganisms to hide the iron micronutrient components around the rhizosphere so that pathogenic microorganisms cannot access it (Pecoraro *et al.,*2022)

It is suspected that this consortium contains several microorganisms that are good for agriculture to increase the productivity and growth of corn plants. Like (Miljakoviæ *et al.,* 2024), plant nutrients may also contribute to the higher efficacy of microbial inoculants as well as the growth and development of plants. Moreover, these organisms exhibit the capability to colonize plant roots, providing numerous benefits to their hosts, including the modulation of phytohormone production, enhancement of soil nutrient availabilityand bolstering resistance against pathogens.

Furthermore, their presence minimizes the reliance on chemical fertilizers, mitigates both biotic and abiotic stress factorsand ultimately boosts crop production, as highlighted (Asghari *et al.,* 2020). Among the microorganisms utilized to augment agricultural productivity are *Azospirillum*, *Arthrobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Flavobacterium*, *Pseudomonas*, *Rhizobium*, *Frankia*, *Klebsiella*, *Clostridium*, *Trichoderma*, *Beauveria*, *Serratia* and *Streptomyces,* as outlined (Jeane and Dias-filho, 2021).

Nitrogen is a vital macronutrient essential for protein and nucleic acid synthesis. Microorganisms like *Azospirillum*, Azotobacter, Achromobacter, Bradyrhizobium, Beijerinckia, Rhizobium, Clostridium, Klebsiella, Anabaena, Nostocand Frankia play a significant role as biological nitrogen fixers, converting atmospheric nitrogen gas (N2) into ammonia (NH3) (Bhat *et al.,* 2019). This bacterial consortium possesses the ability to assimilate nitrogen-fixing microorganisms, facilitating nutrient recycling, particularly for phosphorwiSus (P) and potassium (K), while also stimulating plant growth. The presence of nitrogen (N) and phosphorus (P) components fulfils the nutritional requirements of plants. The P component is essential for plants to affect photosynthesis and determine harvest age. Throughout the process of seed filling, seeds assimilate nutrients from the leaves and other plant tissues. These acquired nutrients serve as essential components for a multitude of metabolic pathways, encompassing protein synthesis, starch formationand other biochemical processes vital for seed development. Concurrently, seeds undergo notable metabolic transformations, including the activation of enzymes engaged in starch metabolism, facilitating the storage of energy in the form of starch. As seeds reach maturity, there is an increased accumulation of metabolic by products, contributing to the attainment of maximum size and weight in the produced seeds (Shaw and Cheung, 2021).

The number of leaves and plant height were not affected by those treatments. This is presumably because internal factors influence the number of leaves. In line with research (Herhandini *et al.,* 2021), the provision of rice husk charcoal and the consortium bacteria does not affect the number of leaves on corn plants because plant leaves are more due to plant genetics. According to (Jeane and Dias-filho, 2021), components affecting plant growth, the number of leaves is genetically determined by abiotic factors such as soil (nutrients, heavy metals, pH and salinity), water availability, light intensityand temperature can affect plant-microbe interactions, as they can alter plant metabolism, root exudate composition and rhizosphere biology. This is because, if changes in plant exudative patterns occur, the same treatment and the same plant genotype may interact differently.

CONCLUSION

The intensity of downy mildew attack on the combined application of the bacterial consortium and rice husk charcoal was lower compared to no combination treatment. The length and diameter of plants treated with the rice husk charcoal plants had a significant effect. Still, the application of the bacterial consortium had no effectand the number of leaves was not affected by the treatment. To control downy mildew disease, 15 tons/ha of husk charcoal can be used in combination with consortium bacteria applied to leaves and soil.

Conflict of Interest

All authors declare that they have no conflicts of interest.

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