Indonesian Journal of Agronomy

ISSN 2085-2916 | e-ISSN 2337-3652
Indonesian J. Agron., *51*(1), 101-108, April 2023
DOI: https://dx.doi.org/10.24831/ija.v51i1.41697

Research Article

Application of *Trichoderma sp*. and PGPR for preventing downy mildew incidence on sweet corn

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ABSTRACT

Downy mildew is a disease caused by the fungus Peronosclerospora maydis. Application of biological agents to control downy mildew is a kind of environmentally friendly control method, that widely promoted recently. Trichoderma sp. and plant growth promoter rhizobacteria (PGPR) are root symbionts, which can induce plant resistance against disease infection. This study aimed to determine the effective application of Trichoderma sp. combined with various concentrations of rhizobacteria to control downy mildew. This study used a completely randomized design (CRD) method with 6 treatments and 5 replications. The treatment was the concentration of rhizobacteria consisting of P0 (control) = without treatment, P1= 15 mL L^{-1}. P2 = 30 mL L^{-1}. P3 = 45 mL L^{-1}. P4 = 60 mL L^{-1} and P5 (positive control) = seed treatment with 5 g kg^{-1} dimetomorf fungicide. Each rhizobacteria suspension was mixed with Trichoderma sp. solution as much as 15 mL L^{-1}. The parameters observed consisted of plant height (cm), number of leaves, disease incidence (%), and disease severity (%). 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 severity of the disease up to 89.84%.

Keywords: bacteria, concentration, downy mildew, sweet corn, *Trichoderma* sp.

Edited by:
Willy B. Suwarno

Received:
26 June 2022
Accepted:
14 April 2023
Published online:
27 April 2023

Citation:
Purwanti, E. W., Wahyudi, D., & Hamyana. (2023). Application of *Trichoderma* sp. and PGPR for preventing downy mildew incidence on sweet corn. *Indonesian Journal of Agronomy, 51*(1), 101-108

INTRODUCTION

Sweet corn (*Zea mays sacharatta* L.) is a horticultural commodity that has the potential to be developed in Indonesia. Sweet corn can be harvested young, and the production time is much shorter than grain maize. Farmers in Sukoanyar Village, Pakis Subdistrict, Malang, have widely cultivated sweet corn. According to the Central Board of Statistics Malang Regency (BPS, 2019), sweet corn production in Pakis subdistrict decreased from 2017 (3,079 tons) to 2018 (2,077 tons) to 2019 (937 tons). According to farmers, the cause of production decline is due to downy mildew.

In Indonesia, three pathogenic fungi causing downy mildew on sweet corn are identified, i.e., *Peronosclerospora maydis*, *P. philippinensis*, and *P. sorghi* which spread in different regions (Rustiani et al., 2015). According to Rustiani et al. (2015) *P. maydis* clusters in the Java and Lampung of southern Sumatera, *P. sorghi* clusters in the Medan and Aceh of northern Sumatera, and *P. philippinensis* clusters in the Gorontalo northern Sulawesi.

Based on preliminary research, the downy mildew case in Sukoanyar Village of Malang is identified as *P. maydis*. The infection has typical symptoms of leaf chlorosis extending parallel to the veins with white to yellow stripes and clear boundaries. Infected plants show stunted growth, and show a white powdery layer under the leaf surface. The fungus *P. maydis* infects plants aged 2-3 weeks with systemic symptoms in the whole plant noticeable when the infection reaches the maize growing point. According to Daryono et al. (2018) and Ridwan et al. (2015) sweet corn infected by *P. maydis* at growing points is

unable to produce cobs and the downy mildew causes a decreasing in cob production up to 100%.

Until recently, efforts have been made to control downy mildew by using a fungicide with active ingredients dimethomorph or metalaxyl. However, continuous use of the chemical application is not recommended because it has a higher risk of developing oomycete *Peronosclerospora* resistance (Anugrah et al., 2016). Therefore, alternative biological control is developed using antagonistic microbes such as *Trichoderma* sp. and Plant Growth Promoting Rhizobacteria (PGPR). Unlike *Trichoderma* sp., an antagonistic fungus, PGPR is a consortium of bacteria that are active in colonizing plant roots with a function of increasing plant growth, soil fertility, yields, and a disease control agent.

Many microorganisms from the class of bacteria and fungi have antagonistic effects on particular diseases can be utilized as biological agents. For example, Patchouli plants formulated with *Pseudomonas fluorescens* were able to reduce disease intensity from 72.5% to 16.5-24.1% (Sivasakthi et al., 2014). The combined application of *Trichoderma* sp. and *P*. *fluorescens* bacteria can also suppress clubroot disease in *Brassica oleracea* L. into zero infection. The case of zero infection was due to *Trichoderma* sp. having activity as a mycoparasitic, antibiosis, and nutritional competitor (Pradnyana et al., 2018). Based on this such experience, it is necessary to conduct further studies on the effectiveness of the application of *Trichoderma* sp. with various PGPR concentrations for downy mildew control in sweet corn. This study aimed to determine the effective application of Trichoderma sp. combined with various concentrations of rhizobacteria to control downy mildew.

MATERIALS AND METHODS

The research was carried out in the university land belonging to the Agricultural Development Polytechnic of Malang (Politeknik Pembangunan Pertanian Malang), East Java. Propagation of *Trichoderma* sp. and PGPR was carried out at the Laboratory of Plant Protection, Department of Agriculture, Politeknik Pembangunan Pertanian Malang. The research was conducted from January to April 2022.

Research design

The study used a completely randomized design with 6 treatments and 5 replications. There were 2 control treatments, namely P0 as a negative control and P5 as a positive control. The positive control was a preventive treatment of downy mildew through seed treatment using a fungicide, while the negative control was no treatment or no disease control intervention. The treatments were a mixture of 50 mL L^{-1} *Trichoderma* sp. suspension with various levels of PGPR suspension, including 15, 30, 45, and 60 mL L^{-1}. Observational data were analyzed statistically using analysis of variance (ANOVA) at a level of $\alpha = 5\%$ and continued with the Duncan Multiple Range Test (DMRT) at a test level of 5%.

The materials used sweet corn seeds of F1 Talenta Pertiwi variety, suspension of cultured *Trichoderma* sp., suspension of cultured PGPR, downy mildew spore inoculum, water, distilled water, urea, NPK fertilizer (16:16:16), manure as base fertilizer, fungicide with dimethomorph active ingredients, and 70% alcohol. The research tools included Potassium Permanganate solution (1%), glass wool, an air pump, a 5 mm diameter hose, a digital balance, a sprayer, a hemocytometer, a binocular microscope, 50 cm x 50 cm polybags, measuring tape, and camera.

Propagation of Trichoderma sp.

Propagation of *Trichoderma* sp. was carried out with the following work steps; EKG media materials were prepared, containing 20 L of water, 6 kg of potatoes, and 0.5 kg of sugar. Potato was sliced 2 cm x 2 cm x 2 cm, washed, and then put in a pot of water. Sugar was added to the stew pot, then heated to 100 ^{o}C for 60 minutes.

The *Trichoderma* isolate was put into a gallon containing EKG media and fermented for 21 days. Calculation of the density of *Trichoderma* sp. spores is using a hemocytometer, carried out in the Laboratory of Plant Protection, Polbangtan Malang. The test results showed that the density of the suspension of *Trichoderma* sp. was 1.12×10^7 spores mL^{-1}.

PGPR suspension propagation

PGPR suspension was propagated in steps as follows; starter was collected by soaking 100 g of *petung* bamboo roots in 1 liter of sterilized water for 2 days. The soaking water will be used as a source of bacteria. Marthin et al. (2020) stated that bamboo root extract contains bacteria of *Pseudomonas* sp., *Bacillus* sp., and other rhizobacteria.

Media was prepared by mixing 100 g of shrimp paste, 0.5 kg of rice bran, 200 g of sugar, and 15 g of lime into boiling water with a volume of 20 L. A homogeneous mixture was boiled until boiling for 60 minutes. The solution was cooled to room temperature, then 1 L of bacterial growth was added. Then the solution was fermented for 21 days. The result of calculating the bacterial density of the PGPR fermented suspension was 5.16×10^7 cfu mL^{-1}.

Preparation of planting media and maintenance

Media was prepared by sterilizing the soil using the drying method in a Greenhouse for 3 days and spraying 5 L of 70% alcohol, to eradicate the contamination of pathogenic microbes. The ratio of planting media was sterile soil, manure, and husk charcoal with a ratio based on a volume of 2: 1: 1. The spacing used 50 cm x 20 cm. Maintenance included regular watering in the morning and evening. Follow-up fertilization was carried out at 10, 20, and 30 days after planting (DAT) with a dose of 3 grams per polybag of NPK fertilizer (16:16:16).

Application of Trichoderma sp. and PGPR

The seeds were cleaned from the factory fungicide with aquadest. Clean sweet corn seeds were soaked in a solution according to the concentration of the PGPR treatment for 12 hours.

One seed was planted in a polybag and then covered with manure. Suspension *Trichoderma* sp. and PGPR according to the treatment were sprayed in the growing point area of the plants every week starting 7 DAT.

Spore suspension preparation and P. maydis inoculation

Downy mildew inoculums were taken from symptomatic corn leaves taken from nearby fields. Leaves with downy mildew symptoms were soaked in a 5% sugar solution for 5 hours. Spraying the inoculation of the *P. maydis* fungus was carried out in the early morning at 00.30 western times of Indonesia, as much as 5 ml per plant when it was 1 week after planting (WAP).

Observation parameters

Plant height and number of leaves were observed every week from 1 to 6 WAP. Plant height was measured by using a tape measure and the number of leaves was calculated based on the leaves that have fully bloomed. Plant disease incidents were observed at 7, 14, 21, 28, and 35 days after inoculation (DAI). Disease incidence is calculated using the formula of disease incidence (Sekarsari et al., 2013):

$DI = \frac{A}{B} \times 100\%$, where DI = Incidence of downy mildew (%), A = Number of infected sweet corn plants, B = Total observed sweet corn plants.

Disease severity was observed at 7, 14, 21, 28 and 35 DAI. The calculation is based on the area of chlorotic symptoms on the leaf surface. The formula used to calculate the severity of the disease is as follows:

$S = \frac{\sum(ni \ x \ vi)}{v \ x \ z} x$ 100%; where S = Severity of downy mildew (%), ni = Number of leaves per attack category I, vi = Scale value of each attack category I, V = The highest category scale value, Z = Number of leaves observed. The disease severity scale score category according to Matruti et al. (2013) is presented in Tables 1 and 2.

Table 1. Downy mildew disease severity scale.

Damage scale	Description of damage	Percentage of infection
0	No damage	0%
1	Light	> 0–25%
2	Moderate	>25–50%
3	Heavy	>50–75%
4	Totally damage	>75–100%

Table 2. Scale of resistance to downy mildew.

Disease intensity (X)	Resistance scale
0	Totally resistant
0 < X < 25	Resistant
25 < X < 50	Moderate
50 < X < 75	Susceptible
75 < X < 100	High susceptible

RESULTS AND DISCUSSION

Plant height

The results of the analysis showed that the application of a mixture of *Trichoderma* sp. with various concentrations of PGPR significantly affected plant height (Table 3). Based on Table 3 the best results were in the 60 mL L^{-1} PGPR application treatment with an average plant height of 155.59 cm and the lowest value was shown in the control treatment with an average of 106.59 cm.

Table 3. The average height (cm) of sweet corn plants due to treatment.

Treatment	Observation					
	1 WAP	2 WAP	3 WAP	4 WAP	5 WAP	6 WAP
Control	6.99a	14.99a	26.40a	45.53a	84.99a	106.59a
Trichoderma sp. +PGPR 15 mL L^{-1}	7.53a	15.26a	28.73ab	48.13ab	94.93b	129.86b
Trichoderma sp. +PGPR 30 mL L^{-1}	7.72a	15.7ab	29.39b	49.79bc	95.92b	131.26b
Trichoderma sp. +PGPR 45 mL L^{-1}	8.73b	16.66bc	31.86c	52.73c	106.39c	149.52c
Trichoderma sp. +PGPR 60 mL L^{-1}	9.19b	17.46c	33.06c	53.19c	108.79c	155.59c
Dimetomorf (5 g kg^{-1})	7.31a	15.13a	27.53ab	46.86ab	85.66a	115.79a

Note: WAP: weeks after planting; Mean values followed by the same letter were not significantly different based on Duncan's Multiple Range Test at $p < 0.05$ significance level.

It was stated by Khoiri et al. (2021), that *Bacillus* spp. able to increase plant height up to 32.7%. The increase in plant height was due to the role of *Bacillus* sp. as a growth booster (PGPR). *Bacillus* spp. is also capable of producing phytohormone precursors (IAA), producing siderophores, and dissolving phosphates (Sivasakthi et al., 2014; Kashyap et al., 2019). Also reported by Kafrawi et al. (2015), PGPR can increase plant growth through several mechanisms, including producing phytohormones, encouraging nutrient absorption by increasing the availability of N and P nutrients and increasing the solubility of organic and inorganic phosphates. The hormone produced by the PGPR

bacteria is presumably a responsible factor for increasing vegetative growth in plant height. However, it needs further study.

Number of leaves

The results of statistical analysis showed that the application of a mixture of *Trichoderma* sp. with various concentrations of PGPR significantly affected the increase in the number of leaves (Table 4). The best result was shown in the 60 mL L^{-1} PGPR application treatment with an average number of leaves of 14.66 and the lowest value was shown in the control treatment with an average of 11.33. *Trichoderma* sp and PGPR application treatment can trigger an increase in leaf number by up to 29%. According to Pradhipta et al. (2019), the process of introducing PGPR triggers the addition of hormones, both cytokinins and gibberellins. The existence of this hormone indirectly accelerates vegetative growth because it triggers an increase in the number and size of leaf chlorophyll cells.

Table 4. The average number of leaves of sweet corn plants due to treatment (strands).

Treatment	Observation					
	1 WAP	2 WAP	3 WAP	4 WAP	5 WAP	6 WAP
Control	2.33a	4.19a	6.66a	8.46a	10.26a	11.33a
Trichoderma sp. +PGPR 15 mL L^{-1}	2.39a	4.53a	6.86a	8.99ab	11.93b	12.66b
Trichoderma sp. +PGPR 30 mL L^{-1}	2.66ab	5.39b	7.59b	9.26ab	11.99b	12.73b
Trichoderma sp. +PGPR 45 mL L^{-1}	2.86b	5.59b	7.66bc	9.73b	12.93c	13.93c
Trichoderma sp. +PGPR 60 mL L^{-1}	3.06b	6.00b	8.06c	10.53c	13.53c	14.66d
Dimetomorf (5g kg^{-1})	2.39a	4.33a	6.73a	8.66a	10.53a	12.46b

Note: WAP: weeks after planting; mean values followed by the same letter were not significantly different based on Duncan's Multiple Range Test at $p < 0.05$ significance level.

Disease incidence

The results of statistical analysis showed that the application of *Trichoderma* sp. with various PGPR concentrations had a significant effect on reducing the incidence of downy mildew (Table 5). The susceptible phase to systemic attack of downy mildew is at the age of plants up to 14 DAI. In this phase infection often occurs at the growing point.

Table 5. The average incidence of sweet corn plant diseases due to treatment (%).

Treatment	Observation				
	7 DAI	14 DAI	21 DAI	28 DAI	35 DAI
Control	66.66a	73.33a	86.66a	86.66a	86.66a
Trichoderma sp. +PGPR 15 mL L^{-1}	33.47b	33.47b	46.80b	46.80b	46.80b
Trichoderma sp. +PGPR 30 mL L^{-1}	26.94b	33.61b	40.13b	40.13b	40.13b
Trichoderma sp. +PGPR 45 mL L^{-1}	26.94b	26.94b	33.61b	40.27b	40.27b
Trichoderma sp. +PGPR 60 mL L^{-1}	26.94b	26.94b	26.94b	33.47b	33.47b
Dimetomorf (5g kg^{-1})	0.70b	7.22b	13.75b	13.75b	13.75b

Note: DAI: days after inoculation; Mean values followed by the same letter were not significantly different on Duncan's Multiple Range Test at $p < 0.05$ significance level.

In the experimental field, the application of *Trichoderma* sp. and PGPR was able to match the fungicide effect of dimethomorph, with inhibition of disease incidence reaching 36.7 to 45.5% compared to controls. The lowest disease incidence, which amounted 13.75%, was obtained in the dimethomorph fungicide treatment (Table 5). The occurrence of downy mildew symptoms on plants with dimethomorph fungicide was very low compared to the negative control. This is in accordance with research by Anugrah et al. (2016) that fungicides with active dimethomorph ingredients are very effective and can damage downy mildew conidia up to 39.99%. In the local infection phase, namely after 21

DAI, the application of *Trichoderma* sp. and PGPR was able to inhibit disease events from 31 to 54% compared to controls.

The defense mechanism of *Trichoderma* sp. and PGPR have been widely reported to overcome downy mildew. Khoiri et al. (2021) stated that several *Bacillus* isolates could reduce the incidence of downy mildew by up to 80.85%. One type of PGPR bacteria, namely *Pseudomonas fluorescens* has the potential to inhibit the spread of anthracnose disease in chili plants by degrading pathogenic fungal cells and producing antibiotics (Permatasari et al., 2016; Sivasakthi et al., 2014). Harni et al. (2017) stated that the secondary metabolites of *Trichoderma* spp. acts as a potential toxin or antifungal in controlling vascular strake dieback (VSD) in cocoa seedlings, which is caused by the fungus *Oncobasidium theobroma*. Another potential possessed by the fungus *Trichoderma* spp. it is also able to decompose organic matter and strengthens the root system, this causes nutrient availability to increase (Ivayani et al., 2018).

Disease severity

The results of statistical analysis showed that the application of *Trichoderma* sp. with various concentrations of PGPR, showed a significant effect on reducing disease severity due to downy mildew (Table 6). The effect of reducing the severity due to treatment matched the effect of dimethomorph fungicide. The rate of reduction in severity ranged from 34 to 50% compared with the susceptible control (up to 14 DAI). In the next phase, the percentage of disease severity reduction reached 26 to 43% compared to controls.

Table 6. Average disease severity of sweet corn plants due to treatment (%).

Treatment	Observation				
	7 DAI	14 DAI	21 DAI	28 DAI	35 DAI
Control	4.52a	9.24a	18.65a	26.20a	38.56a
Trichoderma sp. +PGPR 15 mL L^{-1}	1.87b	4.65b	7.63b	12.98b	16.44b
Trichoderma sp. +PGPR 30 mL L^{-1}	1.87b	4.32b	6.70b	9.84b	14.47b
Trichoderma sp. +PGPR 45 mL L^{-1}	1.56b	3.41b	5.69b	8.86b	12.23b
Trichoderma sp. +PGPR 60 mL L^{-1}	1.36b	3.16b	4.34b	7.51b	10.16b
Dimetomorf (5g kg^{-1})	0.70b	1.09b	2.59b	3.90b	5.61b

Note: DAI: days after inoculation; Mean values followed by the same letter were significantly different based on Duncan's Multiple Range Test at $p < 0.05$ significance level.

The highest resistance due to PGPR induction was found in the *Trichoderma* sp. +PGPR 60 mL L^{-1} treatment with a severity level of 10.16% (Table 6). Control plant produced the lowest disease resistance with a severity level of 38.56%. The role of PGPR is very close to suppressing the severity of downy mildew because *P. fluorescens* bacteria are antagonistic to pathogens. The antagonistic mechanism of *P. fluorescens* as stated by Khaeruni et al. (2013), is faster colonization. This happens because PGPR has higher adaptability in plant roots so it is more efficient in utilizing various substrates as a source of nutrition. Mixed application of *Trichoderma* sp. with various concentrations of PGPR is able to reduce disease severity from mildly resistant conditions (in controls) to resistant with disease severity of less than 25%.

The potential antagonist of *Bacillus* sp. as a component of PGPR was also observed by Khoiri et al. (2021), which stated that these bacteria were able to suppress the development of downy mildew disease by up to 82.71%. Bilginturan et al. (2021) explained that the mechanism of plant resistance is related to the formation of peroxide enzymes, polyphenol oxidases, phenylalanine ammonium lyase, and β-1.3 glucanase which are antagonistic to pathogens.

CONCLUSIONS

Mixed application of *Trichoderma* sp. with various PGPR concentrations had a significant effect on plant height and the number of leaves. A mixed application of

Trichoderma sp. and PGPR bacteria was effective in inhibiting disease incidence by up to 66.53% and disease severity by up to 89.84% in sweet corn.

ACKNOWLEDGMENTS

We thank Dr. Lisa Navitasari SP., MP. and Iqomatus Sa'diyyah, SST. from the Plant Protection Laboratory of the Politeknik Pembangunan Pertanian Malang, for their contributions to this study.

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