International Journal of Agricultural Industry and Food Technology
JAIFTECH
journal homepage: https://jaiftech.sinergis.org/

Article

Controlling of Downy mildew (*Perenosclerospora maydis*) in Pulut Maize using Biological Agents *Bacillus subtilis* and *Pseudomonas fluorescens*

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Citation: Hidayah, S. N., ALif, T., Aisyah, M. D. N., Erdiansyah, I., Widodo, T. W., & Nuraisyah, A. (2025). Controlling of Downy mildew (*Perenosclerospora maydis*) in Pulut Maize using Biological Agents *Bacillus subtilis* and *Pseudomonas fluorescens*. *International Journal of Agricultural Industry and Food Technology*, *1*(2), 52-57. https://doi.org/10.37148/jaiftech.v1i2.10

Received: March 19, 2025
Revised: May 25, 2025
Accepted: May 27, 2025
Published: June 24, 2025

Abstract: Downy mildew is the most common disease affecting maize crops in Indonesia. An alternative control being developed is the use of biological agents. This study was to determine the effectiveness of *B. subtilis* and *P. fluorescens* against downy mildew on pulut maize plants. This study uses Factorial Randomize Block Design (FRBD). The first factor being the combination of bacteria its J1 (*Bacillus subtilis* (5 ml/l) + *Pseudomonas fluorescens* (20 ml/l)), J2 (*Bacillus subtilis* (10 ml/l) + *Pseudomonas fluorescens* (35ml/l)). The second factor was the application time, its T1 (2 applications), T2 (3 applications) The parameters observed in this study were spore identification, disease incidence, disease intensity and cob weight. The results showed that *B. subtilis* and *P. fluorescens* had no effect on downy mildew attack and yield increase with disease incidence of 90%, 10% disease intensity and yield (cob weight) of 3362.1 grams.

Keywords: Downy mildew; Bioagents; Control

1. Introduction

Pulut maize is a variety of maize that is not widely planted but has recently been developed in Indonesia. It is softer than conventional maize and has a unique sticky rice-like flavor. In addition, it has a short production period of 60 to 70 days after planting (DAP), making it very profitable. However, pulut maize has the disadvantage of being susceptible to pests and diseases. One of the most common diseases is downy mildew.

Downy mildew is a serious disease of maize caused by the pathogen *Perenosclerospora maydis*. The pathogen *P. maydis* infects maize for 2- 4 weeks, causing about 95% damage. Common symptoms of downy mildew in maize are chlorosis that spreads lengthwise parallel to the leaf veins, stunted plant growth, and a white powdery coating visible under the leaf surface in the morning. In severe cases, the plant may die completely (Kurniawan, 2017).

Many control efforts have been made to eradicate this disease. To combat downy mildew, farmers usually turn to synthetic chemical pesticides. This is because while this method is effective relatively quickly, its long-term use can have a negative impact on the environment (Kurniawan, 2017). Chemical control can also result in resistance to the pathogen *P. maydis*, air pollution, and agricultural crops, ultimately resulting in human health problems (Utomo et al., 2020). Given this, it is necessary to develop new innovations as an alternative to environmentally friendly pest control, through of biological control.

The advantage of controlling pests and disease with biological control is that the microorganisms used are still alive and able to multiply, so that effectiveness in the field can be sustained over a long period of time. One group of PGPR microorganisms include

B. subtilis and P. fluorescens which can fight the fungus *P. maydis*, and are known to produce siderophores that are effective in controlling pathogenic microorganisms (Sudewi, 2020). These two bacteria act as biofertilizers, biostimulants, and bioprotectants, which provide positive benefits for plants. When applying *P. fluorescens* and *B. subtilis* on plants, proper timing is required for optimal results. The application time will vary, depending on how long the bacteria have been around. With the right application time, antagonistic bacteria can reach optimal environmental conditions such as humidity, temperature, and critical periods when pathogens usually attack plants (Anjarwati and Aini,2020).

This study aims to determine the effectiveness of biological agents, in this case *B. subtilis* and *P. fluorescens* to prevent downy mildew attack on maize plants.

2. Materials and Methods

This research was conducted from July 12 to Deember 30, 2024 at the experimental field and plant protection laboratory of Politeknik Negeri Jember. The materials used in the cultivation area were glutinous maize seeds of Arumba variety, urea fertilizer, SP- 36, Phonska, KCL, manure, *Pseudomonas fluescens* and *Bacillus subtilis* obtained from LPHTP Tanggul, Jember and PHT Kediri. Materials used in the laboratory included microscope, methylene blue, tape and plants infected with downy mildew (*P. Maydis*) collected from Kebonsari field, and labels.

This study used a factorial randomized Block design (FRBD) with the first factor is the combination of bacteria, J1 (*Bacillus subtilis* (5 ml/l) + *Pseudomonas florescens* (20 ml/l)), J2 (*Bacillus subtilis* (15 ml/l) + *Pseudomonas flourescens* (50 ml/l)) The second factor is the application time, T1 (applied twice), T2 (applied 3 times) with 3 replications. Data were analyzed using the SPSS program by conducting an analysis of variance (ANOVA) test and then conducting further tests with Duncan (DMRT) at the 5% level.

2.1. Identification of Perenosclerospora maydis Spores

Taking spores on symptomatic leaves in the research field with solatip, left for 2 minutes. The collection was done in the morning at 05.30 WIB when the leaves were still dewy. Solatip leaves were observed under a microscope.

2.2. Application of Bacteria B. subtilis and P. fluorescens

Bacterial application for the T1 treatment is once every 1 week. In the T2 treatment every 2 weeks. The application is done by spraying on the area around the plant with the concentration tested for each combination and is done in the morning.

2.3. Parameters Observation

Parameters observed in this study included plant height, disease incidence (KP) , disease intensity (IS), and cob weight. At harvest, the cob weight was determined by weighing each treatment without removing the maize husks. Observation of the incidence of downy mildew was carried out every week from the age of 2- 6 weeks after planting, using the formula of Zainuddin et. al (2014):

$$KP = \frac{n}{v} \; x \; 100\%$$

Description:

KP: Disease incidence (%) n : Number of diseased plants v : Number of plants observed

Disease intensity was observed weekly from 2-6 weeks of age using the formula:

$$IS = \frac{\sum (n \ x \ v)}{N \ x \ Z} x \ 100\%$$

Description:
IS : Disease Intensity
n : Number of diseased leaves in each disease category
v : Scale value of each disease category
N : Number of leaves observed
Z : The highest scale of the disease category

Table 1. Disease categories (Khoiri et al., 2021)

Score	Disease category
0%	Normal
>0-25%	Mild
>25-50%	Moderate
> 50 - 75%	Severe
>75 - 100%	Ver heavy attack

2.4. Data Analysis

The data obtained in this study will be analyzed statistically using the ANOVA test method in the SPSS 30 program and then further tested using the Duncan Multiple Range Test (DMRT) test at the 5% level.

3. Results and Discussion

3.1. Identification of P. maydis

Microscope observations showed that the . *P. maydis* has stalk-like conidiophores and spores are located on the stem.stalk tip. In addition, the *P. maydis* also has round and slightly elliptical conidia. This is reinforced by research (Ali and Kasiamdari, 2024; Trisnawati, 2017; Windriyati, 2024) which states that the conidiophores of *P. maydis* resemble stems and have spores arranged in groups at the ends of the branches, as well as conidia that are round elliptical.

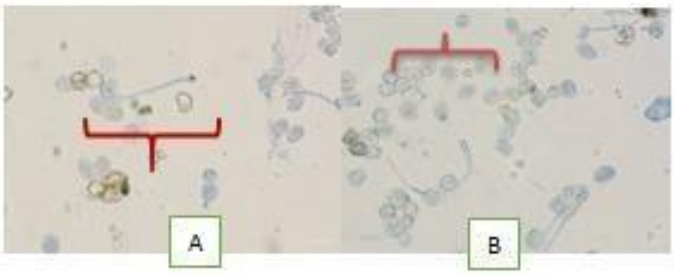

Figure 1. Microscopic observation of P. maydis (A) Conidia shape), (B) conidiophore shape

Observation in the field, plants infected with downy mildew show symptoms, the presence of a white powdery coating under the leaf surface which causes chlorosis. Chlorosis is characterized by the presence of pale lines parallel to the leaf veins. This finding in line with the explanation given by Ridwan et al. (2015) which reveals that the initial symptoms of downy mildew are visible in the form of lines (chlorosis) parallel to the leaf veins, then chlorosis spreads to all parts of the leaf surface.

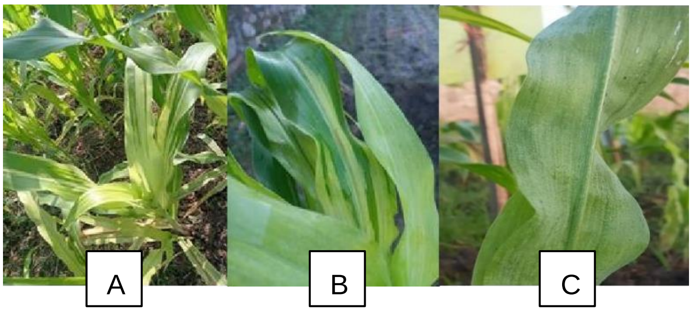

Figure 2. Macroscopic observation of P. maydis symptoms (A), (B) chlorotic symptoms on the leaves (C) powdery mildew under the leaves

3.2. Disease incidence of P. maydis

Disease incidence is the level of downy mildew attack on maize (Ulhaq & Masnilah, 2019). Based on the results of observations during 42 HST (Table 2), it is known that the use of the combination and application time of *B.subtlis* and *P. fluorescens* did not affect the incidence of downy mildew on maize plants. downy mildew, the development of disease incidence increases every week due to downy mildew (P.maydis) attack on the leaves resulting in damage that cannot fully heal. This factor is influenced by how quickly the pathogen infection takes place, which depends on how resistant the variety is, the availability of downy mildew inoculum sources (conidia), and environmental conditions such as temperature, humidity, and the presence of stagnant water on the lower part of the maize plant after rain (Ali and Kasiamdari, 2024).

Table 2. Average incidence of downy mildew (P.maydis)

Type of bacteria (B.subtilis+P. flourescens)	2MST	3MST	Incidence (%) 4MST	5MST	6MST
J1 (5ml +20ml)	23.08a	38.09a	59.52a	78.57a	88.09a
J2 (10ml + 35ml)	19.05a	42.85a	54.76a	90.47a	90.47a
J3 (15ml+50ml)	19.04a	35.71a	52.38a	83.33a	90.47a
Application					
T1(2 application)	26.98a	53.96a	71.42a	87.30a	88.87a
T2(3 application)	14.28a	36.50a	55.55a	80.95a	85.71a

Notes: Numbers followed by the same letter in each column show no significant difference based on Duncan's test at $P < 0.05$.

3.3. Disease intensity of P. maydis

Table 3. Disease intensity of P.maydis

Type of bacteria (B.subtilis+P. flourescens)	2MST	3MST	Intensity (%) 4MST	5MST	6MST
J1 (5ml +20ml)	1.93a	4.45a	6.53a	8.06a	8.35a
J2 (10ml + 35ml)	1.21a	3.68a	6.66a	7.41a	7.70a
J3 (15ml+50ml)	0.85a	5.60a	7.31a	8.58a	8.59a
Time of Application					
T1(2 application)	1.47a	4.58a	6.99a	8.32a	8.51a
T2(3 application)	1.19a	4.57a	6.68a	7.72a	7.91a

Notes: Numbers followed by the same letter in each column show no significant difference based on Duncan's test at $P < 0.05$.

Disease intensity is a parameter to determine the effect of utilizing biological agents as an inhibitor of downy mildew growth. Disease intensity is calculated based on scoring on each plant leaf (Ulhaq & Masnilah, 2019). Based on the results of observations during 42 HST (Table 3), it is known that the use of the combination and application time of *B. subtlis* and *P. fluorescens* does not affect the intensity of downy mildew, the disease intensity continues to increase every week. Based on the research of Susanti et al. (2022), wind direction also affects the high level of attack. Wind direction can give an advantage to airborne pathogens, causing biological agents may not be able to compensate. Wiwik et al. (2013) explain that when pathogen populations are very large, biological agents cannot compete with them. In this situation, although biological agents can dampen some pathogen growth, their effect is not significant enough to reduce the disease intensity.

3.4. Cob weight

Table 4. Cobs weight

Type of bacteria (*B.subtilis+P. flourescens*)	Cobs *weight*
J1 (5ml +20ml)	1.93a
J2 (10ml + 35ml)	1.21a
J3 (15ml+50ml)	0.85a
Time of Application	
T1(2 application)	1.47a
T2(3 application)	1.19a

Notes: Numbers followed by the same letter in each column show no significant difference based on Duncan's test at $P < 0.05$.

The weight of the cob is a paramater of yield observation. Based on observations during harvest at the age of 70 HST (Table 4), it is known that the use of the combination and application time of *B. subtlis and P. fluorescens* did not affect the yield obtained. This occurs because of the relationship between the level of attack and the appearance of the disease. When the attack increases, the yield tends to decrease (Septiana et al., 2021). Conversely, if the level of attack decreases, the yield will increase. Wahyudin et al. (2015) explained that plants that experience disease attacks on the leaves can interfere with the photosynthesis process, which ultimately inhibits seed development. Imperfections in photosynthesis have an impact on reducing carbohydrate formation and blocking the process of cob initiation (Buge et al., 2017).

5. Conclusion

Based on the results of the study, it can be concluded that *Bacillus subtilis* and *Pseudomonas fluorences* have no effect in controlling downy mildew and increasing yields, with a disease incidence of 90.47%, disease intensity of 10% and a cob weight of 3362.14 grams.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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