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CHARACTERIZATION OF *Peronosclerospora maydis* AND ITS EFFECT ON PLANT GROWTH AND DISEASE INCIDENCE UNDER FUNGICIDES TREATMENT ON MAIZE (*Zea mays* L.)

KARAKTERISASI *Peronosclerospora maydis* DAN PENGARUHNYA TERHADAP PERTUMBUHAN TANAMAN DAN KEJADIAN PENYAKIT DENGAN PERLAKUAN FUNGISIDA PADA TANAMAN JAGUNG (*Zea mays* L.)

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Abstract

Downy mildew disease on maize (*Zea mays* L.) is generally caused by fungi from the genus *Peronosclerospora*. This study aims to identify the type of fungi that cause downy mildew in Sleman, to determine the effect of a fungicide on the growth of maize plants, the disease incidence, and severity. Fungi isolates were taken from the maize plantation area in Tirtoadi Village, Sleman. Observation of fungal spores was carried out using a binocular microscope. Application of metamorph (PMD), metalaxyl (PMMe), propineb (PMP), and mancozeb (PMMz) fungicides was carried out when the plants were 7 days after planting (DAP). Maize plants were inoculated with downy mildew spores when the plants were 8 DAP. The parameters measured were plant height, number of leaves, fresh weight, dry weight, disease incidence, and severity. Data were analyzed by ANOVA using SPSS version 23 and tested by Duncan Multiple Range Test (DMRT). The results showed that the fungi causing downy mildew in Sleman was *Peronosclerospora maydis*. Plants treated with mancozeb (PMMz) had the highest growth in plant height, number of leaves, fresh weight, and dry weight, followed by PMP, PMD, and PMMe treatments. The results of the DMRT test showed that the disease incidence and disease severity of PMD, PMMe, and PMP were significantly reduced compared to the *P. maydis* (PM) treatment. Plants with metalaxyl treatment had the highest ability to reduce disease severity by 92.70%, followed by prominent at 78.10%, metamorph 58.66%, and mancozeb 16.79%.

Keywords: Downy mildew; Fungicide; Identification; *Peronosclerospora maydis*; *Zea mays* L.

Abstrak

Penyakit bulai pada tanaman jagung (Zea mays L.) umumnya disebabkan oleh jamur dari genus Peronosclerospora. Penelitian ini bertujuan untuk mengidentifikasi jenis jamur penyebab penyakit bulai di Sleman, mengetahui pengaruh fungisida terhadap pertumbuhan tanaman jagung, kejadian, dan keparahan penyakit. Isolat jamur diambil dari area persawahan Desa Tirtoadi, Sleman. Pengamatan spora jamur dilakukan dengan menggunakan mikroskop binokuler. Pemberian fungisida dimetomorf (PMD), metalaksil (PMMe), propineb (PMP), dan mankozeb (PMMz) dilakukan saat tanaman berumur 7 hari setelah tanam (HST). Tanaman jagung diinokulasi dengan spora bulai pada saat tanaman berumur 8 HST. Parameter yang diukur adalah tinggi tanaman, jumlah daun, berat basah, berat kering, kejadian penyakit, dan tingkat keparahan. Data dianalisis dengan ANOVA menggunakan SPSS versi 23 dan diuji dengan Duncan Multiple Range Test (DMRT). Hasil penelitian menunjukkan bahwa jamur penyebab bulai di daerah Sleman adalah Peronosclerospora maydis. Tanaman dengan perlakuan PMMz memiliki pertumbuhan tinggi tanaman, jumlah daun, berat basah, dan berat kering tertinggi, diikuti dengan perlakuan PMP, PMD, dan PMMe. Hasil uji DMRT menunjukkan angka kejadian penyakit dan keparahan penyakit pada perlakuan PMD, PMMe, dan PMP menurun secara signifikan dibandingkan dengan perlakuan P. maydis (PM). Tanaman dengan perlakuan metalaksil memiliki kemampuan terbaik dalam menurunkan keparahan penyakit sebesar 92,70%, kemudian propineb 78,10%, dimetomorf 58,66%, dan mankozeb 16,79%.

***Kata Kunci**: Bulai; Fungisida; Identifikasi; Peronosclerospora maydis; Zea mays L.*

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INTRODUCTION

Plant disease is a major obstacle in maize production. Maize leaf diseases are commonly caused by fungi including downy mildew with the pathogens of *Peronosclerospora maydis, P. philippinensis*, and *P. sorghi* (Rashid et al., 2013). The symptoms are the appearance of chlorosis on the leaf veins, plant growth becoming stunted, and a white layer like flour appearing under the leaf surface (Ulhaq & Rahmi, 2019). If the downy mildew attack is severe enough, maize cannot produce seeds and the production will decrease (Ridwan et al., 2015). *P. maydis* commonly found in Java and Lampung, *P. sorghi* is commonly found in Medan and Aceh, and *P. philippinensis* clustered in Gorontalo and Sulawesi (Rustiani et al., 2015). However, some reports indicated that *P. sorghi* was also found in Bogor, West Java (Widiantini et al., 2017). According to Adhi et al. (2021), it showed that *P. philippinensis* was found in Kediri, East Java. It shows that its possible changes in the distribution of downy mildew species in Indonesia. There is a spread of the pathogen that causes downy mildew from one area to another. The first step in controlling downy mildew can be done by characterizing the fungi. Characterization can be done by observing morphological characteristics. Through morphological characters, information on the differences between species will be distinguished.

Fungicide application is still one of the most frequently used to prevent disease in plants. Based on how it works, fungicides are divided into two groups, there are systemic fungicides which work by being absorbed by plant organs, such as dimethomorph and metalaxyl, and non-systemic fungicides which work by forming a barrier layer on the plant surface, such as prominent and mancozeb (Mohamed et al., 2018). Research on the use of metamorph and metalaxyl fungicides was carried out by Anugrah and Widiantini (2018) who used the conidia germination method and observed the damage caused by adding a fungicide to conidia, the effect on plant growth was not observed. The results showed that dimethomorphic fungicide treatment at a concentration of 10,000 ppm was still effective in inhibiting the development of downy mildew as indicated by its ability to suppress conidial germination and caused 37.9% conidia damage. The use of metalaxyl had a good suppressive effect as indicated by the low conidia germination of 0.70% and conidia damage which reached 19.91%. Research on the use of prominent fungicides was carried out by Tanzil and Purnomo (2021) which used the seed treatment method. The results showed that the prominent fungicide treatment was able to reduce the intensity of downy mildew attacks, but did not affect plant height. Redityo (2015) researched the use of the mancozeb by spray method. The fungicide suspension was given when *P. maydis* infection was about 50%. The results showed that Mancozeb 5% fungicide application was not significantly effective in controlling *P. maydis*. The efficacy level was found less than 50%. It is suggested that the inefficiency of the fungicide application was due to the high severity of the disease in the first fungicide application, so the disease development was too fast and difficult to control.

This research was conducted to characterize the species of fungi causing downy mildew in Sleman, Yogyakarta. Research conducted by Rustiani et al. (2015), it showed that downy mildew in Yogyakarta was caused by *P. maydis*, with the possibility of changes in the distribution of downy mildew species, it is necessary to re-identify the species that causes downy mildew in Sleman, Yogyakarta. In this research application of metamorph, metalaxyl, propineb, and mancozeb fungicide was done by using the spray method. Fungicide application was sprayed one day before the plants were inoculated with *P. maydis* spores. This research was conducted to determine the effect of fungicides on plant growth, number of leaves, fresh weight, dry weight, disease incidence, and disease severity.

MATERIALS AND METHODS

The research was conducted at the Sawitsari Research Station and Plant Systematics Laboratory, Faculty of Biology, Universitas Gadjah Mada from November 2022 to January 2023. The materials used in this study included *Peronosclerospora maydis* spores, hybrid maize seeds of Pertiwi 6, planting media containing soil, husk charcoal, and manure in a 1:1:1 ratio, methylene blue dye, four types of fungicides with active ingredients of dimethomorph, metalaxyl, propineb, and mancozeb. The fungi spores were taken from a maize plantation in Tirtoadi Village, Sleman. Maize plants that were infected with downy mildew were characterized by the presence of a white powdery coating on the lower surface of the leaf which is a mass of *Peronosclerospora* spp. (Burhanuddin, 2011). The characterization process of the fungi was carried out by taking the white layer on the surface of the leaf slowly with a piece of cellophane tape and placing it on a glass object that has been given one drop of methylene blue dye and observed under a binocular microscope (Widiantini et al., 2017).

Maize plants that will be treated with fungicide were planted in polybags and placed in the greenhouse. The experiment was arranged in a randomized block design with five treatments and five replications each. The first treatment was PMD (*Peronosclerospora maydis* + dimethomorph fungicide), the second treatment was PMMe (*P. maydis* + metalaxyl fungicide), the third treatment was PMP (*P. maydis* + propined fungicide), the fourth treatment was PMMz (*P. maydis* + mancozeb fungicide), and PM (inoculated with *P. maydis* only). Application of every fungicide at a dose of 2 g/L was carried out when the plants were 7 day after planting (DAP). Spores suspensions were obtained from infected maize plants. Spores were collected by cutting off the parts of the leaves that had symptoms of downy mildew (half diseased and half healthy). The leaves were put into a sprayer containing 100 mL of water, and then shaken until the downy mildew spores were released and mixed into the water (Tanzil & Purnomo, 2021; Talanca, 2015). The spores suspension was sprayed on each plant when they were 8 DAP. This method was done at 05.00–06.00 AM (Adhi et al., 2019).

The parameters measured were plant height, number of leaves, fresh weight, dry weight, disease incidence, and disease severity. Plant height data was obtained by measuring the height of the maize plant from the base of the stem to the top of the plant. Measurements were carried out every seven days until 35 DAP. Data on the number of leaves was obtained by counting the leaves that had fully opened and were still attached to the stem of the plant. The number of leaves was measured every seven days until 35 DAP. The fresh weight of maize was carried out by removing each sample plant from each treatment. The dry weight of maize was obtained by removing the moisture content of the maize plant after the plants were air-dried for 1–2 days in the oven at a temperature of 60 °C. Samples were weighed using an analytical balance until the weight was constant. Disease incidence was measured by calculating the percentage of maize plants attacked by downy mildew. Disease severity was measured based on the occurrence of chlorosis symptoms on the leaves. The severity of downy mildew infection was determined based on a scale of 0–4. On a scale of 0, there was no visible presence of chlorotic and downy mildew spores on maize leaves. On a scale of 1 (1–25%) there was white or yellowish chlorotic that extended across the leaf blade, scale of 2 (26–50%) covered more than 1/4 of the leaf, scale of 3 (51–75%) chlorotic more than half of the leaf, and scale of 4 (76–100%) chlorotic appears all over the leaf surface (Matruti et al., 2013; Ulhaq & Rahmi, 2019). The data were analyzed using SPSS with one-way ANOVA and continued with Duncan's test with a significance level of 5% (α= 0.05). The formula for calculating disease incidence and disease severity is as follows (Chiang et al., 2017), disease incidence (%)= $\frac{n}{N} \times 100$. n= number of maize plants attacked by downy mildew, N= number of maize plants observed, disease severity (%)= $\frac{\sum(ni\ x\ vi)}{N\ x\ V} \times 100$. Notes ni= number of leaves affected, vi= severity score value, N= total number of leaves observed, and V= the highest severity score.

RESULTS

The morphological characterization of the conidia and conidiophore isolates is shown in Table 1. The conidiophore had a size of 217.17–340.79 µm, dichotomously branched (3–4 branches), the conidia were spherical to subspherical with a diameter of 14.33–17.43 × 23.23–33.16 µm, and the cell wall was in the form of a single thin layer.

The isolates of fungi causing downy mildew which taken from a maize plantation in Tirtoadi Village. had a morphological character as shown in Figure 1. The results of the characterization of the morphological characters of the fungi in this study were similar to the appearance of *Peronosclerospora maydis*. Based on the characters observed in the research, it shows that the fungi causing downy mildew in Sleman was *Peronosclerospora maydis*, with diagnostic characters in the form of three to four times the number of branches and the shape of the conidia which differentiates it from another species from *Peronosclerospora*.

Table 1. Comparison of the morphological character of downy mildew fungi from Sleman compared to reference species of *Peronosclerospora*

Morphological character	Observation result	Species of *Peronosclerospora* (Rustiani et al., 2015)		
	P. maydis	*P. maydis*	*P. shorgi*	*P. philipinensis*
Conidiophore size	217.17–340.79 µm	111–410 µm	183–300 µm	150–300 µm
Conidiophore branched	3–4 branches	3–4 branches	2 branches	3 branches
Conidia cell wall	Thin wall	Thin wall	Thick wall	Thin wall
Conidia diameters	14,33–17,43 × 23,23–33,16 µm	12–23 µm	10–11 µm	15–40 µm
Conidia shape	Spherical to subspherical	Spherical to subspherical	Oval	Oval

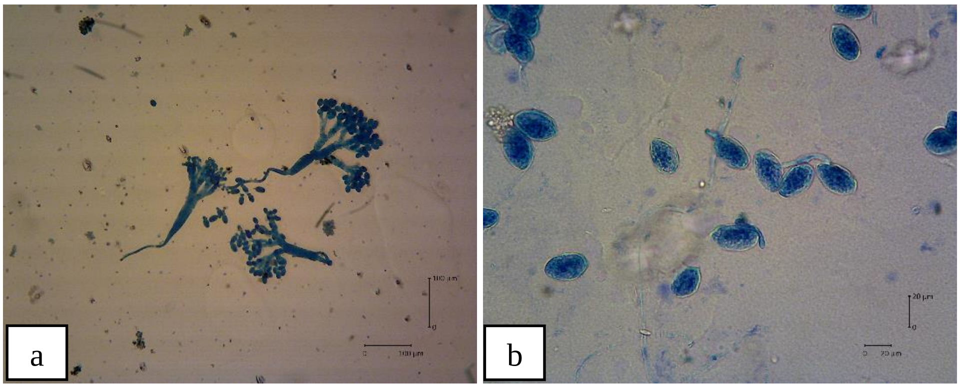

Figure 1. The morphological character of *Peronosclerospora maydis* from Sleman are conidiophore (a) and conidia (b)

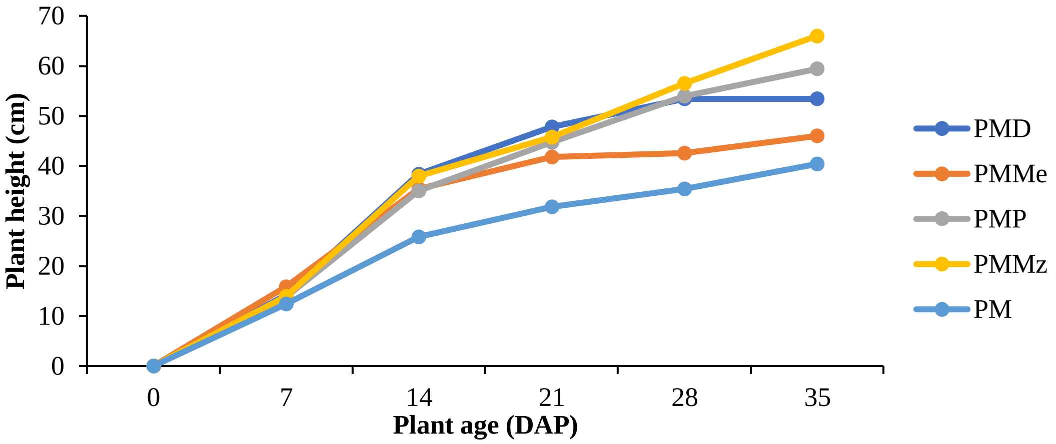

Figure 2. Plant height of *Zea mays* inoculated with *Peronosclerospora maydis* and fungicide treatments from 0–35 days after planting (DAP)

According to Figure 2, the application of fungicide was able to increase plant height. Plants with PMD treatment had plant heights that were not different from those treated with PMP. The growth in height of plants with PMMe treatment was the lowest compared to other treatments. Plants with PMMz treatment had the most optimal and had constant growth until 35 DAP. Plants with PM treatment (without fungicide) had the smallest average growth compared to other treatments.

According to Figure 3, the application of fungicide on the plant that was already inoculated with *P. maydis* affected the number of leaves. Plants with PMD treatment tend to be constant until 28 DAP. Plants with PMMe treatment had an increase in the average number of leaves at 21 DAP. Plants treated with PMP treatment had an average number of leaves that continued to increase until 35 DAP. Plants with PMMz treatment had the highest and most constant average number of leaves compared to other treatments. Plants with PM treatment had the lowest number of leaves compared to other treatments.

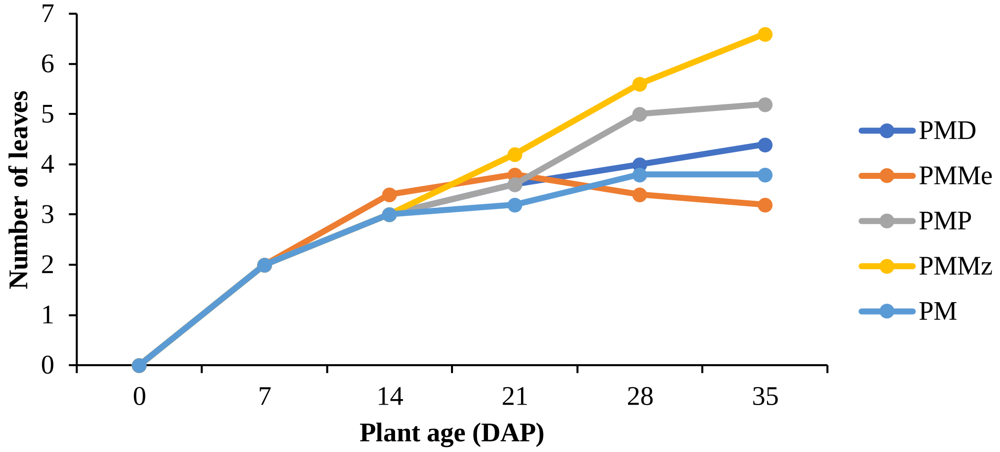

Figure 3. Number of leaves of *Zea mays* inoculated with *Peronosclerospora maydis* and fungicide treatments from 0–35 days after planting (DAP)

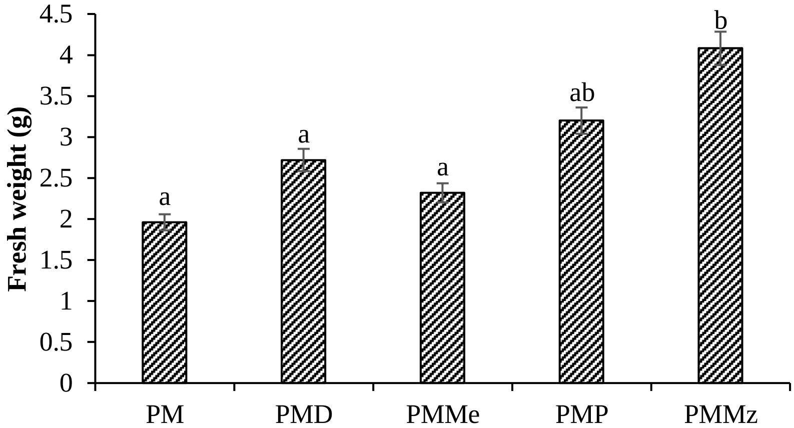

Figure 4. Plant fresh weight of *Zea mays* inoculated with *Peronosclerospora maydis* and fungicide treatments from 0–35 days after planting (DAP); PM= inoculated with *P. maydis* only, PMD= *Peronosclerospora maydis* + dimethomorph fungicide, PMMe= *P. maydis* + metalaxyl fungicide, PMP= *P. maydis* + propineb fungicide, PMMz= *P. maydis* + mancozeb fungicide. The histogram followed by the same letter shows no significant difference at the level of 5% of the DMRT test

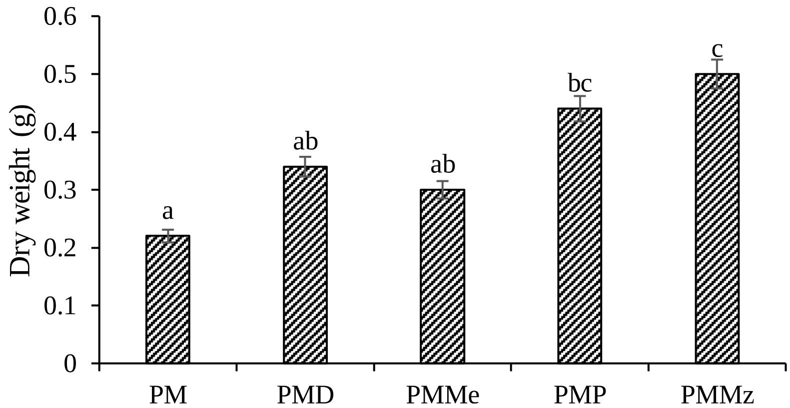

Figure 5. Plant dry weight of *Zea mays* inoculated with *Peronosclerospora maydis* and fungicide treatments from 0–35 days after planting (DAP); PM= inoculated with *P. maydis* only, PMD= *Peronosclerospora maydis* + dimethomorph fungicide, PMMe= *P. maydis* + metalaxyl fungicide, PMP= *P. maydis* + propineb fungicide, PMMz= *P. maydis* + mancozeb fungicide. The histogram followed by the same letter shows no significant difference at the level of 5% of the DMRT test

Figures 4 and 5 showed fungicide treatments had different effects on the fresh and dry weight of the maize plants. The results of the DMRT test showed that the fresh weight of the plant with PMMz treatment was significantly different compared to the PM, which means that the fungicide was able to prevent downy mildew infection. Plant with PMMz treatment has the highest fresh weight value with a value of 4.08 g and the highest dry weight of 0.50 g compared to the other. The results of measuring plant dry weight are directly proportional to the results of measuring plant height and number of leaves.

Table 2. Disease incidence and severity of *Zea mays* inoculated with *Peronosclerospora maydis* and fungicide treatments 35 days after planting (DAP)

Treatment	Disease incidence (%)	Disease severity (%)	Reduction of disease severity (%)
PMD	60.00 ± 0.00^{ab}	14.16 ± 14.89^{ab}	58.66
PMMe	10.00 ± 14.14^{a}	2.50 ± 5.59^{a}	92.70
PMP	30.00 ± 14.14^{a}	7.50 ± 11.18^{a}	78.10
PMMz	50.00 ± 42.43^{ab}	28.50 ± 21.18^{bc}	16.79
PM	100.00 ± 0.00^{b}	34.25 ± 12.42^{c}	0

Note: Values ± SD in the same column followed by the same letter show no significant difference at the level of 5% of the DMRT test

Table 2 showed that fungicide treatments had different effects on the disease incidence and disease severity of the maize. The results of the DMRT test showed that the disease incidence of PMD, PMMe, and PMP was significantly reduced compared to the PM treatment. The results of measuring the percentage of disease incidence showed that PMMe treatment had the smallest percentage of disease incidence which was 10%. Plants with PM treatment had the highest percentage of disease incidence which was 100%. Based on the results of measuring the severity of the disease there was a significant difference between treatments on the percentage of disease severity. The results of the DMRT test showed that the disease severity of PMD, PMMe, and PMP was significantly reduced compared to the PM treatment. PMMe treatment had the smallest percentage of disease severity with a percentage of 2.50%. Plants with PM treatment had the highest percentage of severity with a percentage of 34.25%. Plants with PM treatment had the highest percentage of disease incidence and severity because the plants were not treated with fungicide, all the observed plants were attacked by downy mildew. The PMMe treatment had the highest ability to reduce disease severity by 92.70%, followed by the PMP treatment (78.10%), PMD (58.66%), and PMMz (16.79%).

DISCUSSION

Isolate of *Peronosclerospora maydis* was isolated from a maize plantation in Tirtoadi Village, Sleman. Based on the morphology of conidia and conidiophores, it was found that *P. maydis* was the fungi causing downy mildew in Sleman. *P. maydis* was also reported infected maize (Rustiani et al., 2015; Ulhaq & Rachmi, 2019) and has similar characteristics, the conidiophore had a size of 217.17–340.79 µm, dichotomously branched (3–4 branches), the conidia were spherical to subspherical with a diameter of 14,33–17,43 × 23,23–33,16 µm, and the cell wall was in the form of a single thin layer. The characteristics of *Peronosclerospora* conidia are influenced by climatic conditions, types, and varieties of host plants. The same *Peronosclerospora* species originating from different locations will react differently to the same host plant (Widiantini et al., 2017). The characteristics of *P. maydis* showed differences, compared to *P. shorgi* (Rustiani et al., 2015) and *P. philippinensis* (Cueva et al., 2022; Rustiani et al., 2015).

The application of fungicide was able to increase plant height. Plants with PM treatment (without fungicide) had the smallest average growth compared to other treatments. This was because the PM treatments were exposed to the most severe downy mildew infection so the plants became stunted and did not grow optimally. According to Sutarman, (2017) infection from pathogens has an impact on plant growth, such as hyperplasia, hypoplasia, color changes, dryness, and necrosis. Downy mildew tends to cause plants to become stunted, sterile, dry, and die (Adhi et al., 2019). The more severe the downy mildew infection on the plant, the growth of the plant will be stunted.

The application of fungicide on a plant that was already inoculated with *P. maydis* affected the number of leaves. The number of leaves that did not increase was caused by the leaves that began to dry out caused by downy mildew infection. Dried and dead leaves are triggered by the release of phytoalexins and other secondary metabolites as anti-fungal or anti-bacterial by inducing cell death thereby blocking the spread of adjacent healthy cells (Nookaraju & Agrawal, 2012). In this study, downy mildew infected the leaves. Downy mildew infection occurs in the leaf vessels in the form of chlorotic lines from the base to the tip of the leaf and parallel to leaf reinforcement (Daryono et al., 2018). The more severe the downy mildew infection on the plant, the number of leaves will decrease.

The application of fungicide on plants that were already inoculated with *P. maydis* affect the fresh weight and dry weight of plants. Based on the results, the plant with PMMz treatment had the highest value in growth, which might have caused mancozeb to contain zinc that activates enzymes responsible for the synthesis of certain proteins. Zinc is essential in the formation of auxin, which helps regulate stem growth and elongation (Pthorticulture, 2023). The more severe downy mildew infection, the plants will be stunted and the fresh weight and dry weight will decrease

Based on the effect of fungicide application on plant growth, plants with PMMz treatment had the highest measurement results for plant height, number of leaves, fresh weight, and dry weight, followed by PMP, PMD, PMMe, and PM. Plants with PMMz treatment had the highest value up to 35 DAP. Mancozeb fungicide contains zinc which is essential in the formation of auxin, which helps regulate stem growth and elongation (Pthorticulture, 2023). Plants treated with PM had the smallest average growth compared to other treatments. This was because the PM treatment was exposed to the most severe downy mildew infection, so the plants became stunted and did not grow optimally. Plants with PM treatment had the highest prevalence of disease incidence and severity because the plants were not treated with fungicide, all the plants observed were attacked by downy mildew. Plants with PMMe treatment that contain metalaxyl fungicide have the highest percentage reduction in disease severity, but metalaxyl is still effectively used to prevent the spread of downy mildew. Metalaxyl fungicides are systemic fungicides, that work in a specific way by penetrating fungal cells and selectively interfering with DNA synthesis by inhibiting mycelium growth and spore formation (Tias et al., 2017). Propineb fungicide had the second-highest percentage reduction in disease severity after metalaxyl. Propineb fungicide is a type of fungicide that works in contact (non-systemic). In general, the chance for this type of fungicide to develop resistance to its target is smaller than that of systemic fungicides because contact fungicides are not target-specific, such as systemic fungicides (Bisatani, 2023). Dimethomorphic fungicides had a percentage reduction in disease severity in the third order after prominent. Dimethomorphic fungicides are systemic fungicides that are suitable for protecting plants from attack by the Oomycetes fungi that cause downy mildew (Swibawa et al., 2017). However, the use of dimethomorphic fungicides has decreased effectiveness, the use of a fungicide may be overused so that the pathogen begins to resist. While the fungicide mancozeb had the lowest percentage of reducing the severity of the disease. Mancozeb fungicide is a broad-spectrum contact fungicide that works on many targets or works non-specifically. Mancozeb is a contact fungicide that functions to prevent fungal infections and inhibit the germination of spores that stick to the plant surface.

CONCLUSION

Based on research, it can be concluded that *Peronosclerospora maydis* is the causal agent of downy mildew on maize (*Zea mays* L.). Treatment of plants inoculated with *P. maydis* and mancozeb showed the highest plant growth (plant height, number of leaves, fresh weight, and dry weight) compared to other fungicides, dimethomorph, metalaxyl, and propineb. Treatment with metalaxyl showed the highest reduction of disease severity by 92.70% at 35 days after planting, followed by prominent (78.10%), dimethomorphic (58.66%), and mancozeb (16.79%).

Suggestions that can be given for further research are adding the type of fungicide to test the effectiveness in preventing downy mildew on maize, minimizing external factors that accidentally appear, such as insects found in plants, anatomical and molecular observations of downy mildew can be carried out to see more specifically.

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